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Role of interferon lambda 4 and ALT levels in optimising treatment of HCV for patients with low-stage fibrosis

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ABSTRACT:

The use of new anti-HCV drugs is currently limited by high costs and dual therapy; pegylated interferon and ribavirin (peg-IFN+RBV) still represents the only affordable treatment in patients with low-stage fibrosis. We evaluated the role of Interferon lambda4 (IFNL4) polymorphisms and its combination with on-treatment alanine transaminase (ALT) modification in predicting sustained virological response (SVR) in HCV genotype 1 and 4 patients with low-stage fibrosis. We retrospectively analysed 124 patients with Metavir ≤F2, who received dual therapy at our centre. Genotyping for IFNL4 polymorphisms was assessed at baseline, as well as ALT levels (baseline and week 2, 4, 12 and 24 of therapy). Thirty patients (24%) were TT/TT, 74 (60%) TT/DG and 20 (16%) DG/DG. The SVR rate was significantly higher in TT/TT genotype compare to TT/DG and DG/DG (97% vs. 53% and 50%, respectively, p=0.001). Patients that achieved a 60% reduction of ALT baseline value after 4 weeks of therapy had a significantly higher SVR rate (94% vs. 52%, p<0.001). Factors significantly associated with SVR were TT/TT genotype (p=0.029), RVR (p=0.019) and 60% ALT reduction at 4 week of therapy (p=0.005). The absence of both TT/TT genotype and 60% ALT reduction were negative predictors of SVR (p<0.001). In conclusion, the combined use of IFNL4 polymorphisms and ALT reduction at 4 week of treatment is able to optimize candidates' selection for peg-IFN+RBV, discriminating those that could still benefit from dual therapy from the ones that need the new regimens.

Keywords: Chronic hepatitis C, Sustained virological response, Pegylated interferon, Ribavirin.

ABBREVIATIONS

HCV: Hepatitis C virus; **peg-IFN+RBV**: pegylated interferon and ribavirin; **SVR**: sustained virological response; **DAAs**: direct antiviral agents; **EVR**: early virological response; **IFNL**: interferon lambda; **DG**: delta-G; **ALT**: alanine transaminase; **RVR:** rapid virological response; **NR:** non responders; **REL:** relapsers; **ISG:** interferon-stimulated genes.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health issue with more than 170 millions infected people worldwide[1]. Genotype 1 and 4 account for the majority of chronic HCV infections particularly in the Middle East, North Africa and sub-Saharan Africa[2]. Until 2011, the dual therapy based on pegylated interferon and ribavirin (peg-IFN+RBV) for 48 weeks was the only ap-

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proved treatment for genotype 1 and 4[3], with a sustained virological response (SVR) rate of approximately 50%. The advent of the new direct antiviral agents (DAAs) has radically changed the prognosis of patients with HCV, achieving a SVR rate greater than 90%. For this reason, in current guidelines dual therapy with peg-IFN+RBV has been replaced by new regimens that contain usually one DAA. The recommended regimen to treat naive patients with HCV genotype 1 and 4 and low-stage fibrosis is Sofosbuvir+peg-IFN+RBV for 12 weeks[4]. Unfortunately, for most of the countries, especially the developing ones, the new guidelines are economically unsustainable due to the high costs of the new drugs. In this setting, DAAs availability has been limited to patients with advanced liver disease[5] and peg-IFN+RBV is still the only treatment option for most of the patients. Several predictive factors of SVR have been identified in the last decade, such as early virological response (EVR)[6]. Genome-wide association studies have identified a single nucleotide polymorphism on chromosome 19q13 near the interferon lambda 3 gene (formerly known as IL28B) as variants positively associated with the response to peg-IFN+RBV treatment[7,8]. More recently, a dinucleotide polymorphism (ss469415590) that creates or disrupts an open reading frame in a recently discovered gene, interferon lambda 4(IFNL4), has been showed to be strongly involved in response to antiviral therapy[9,10]. The IFNL4 gene (encoding IFNL4) is situated upstream of interferon lambda 3. The one-base deletion in the delta-G (DG) variant results in a frame shift, which in turn produces the full-length protein designated as IFNL4; the TT variant does not produce IFNL4[11]. The TT/TT variant seems to be associated with impaired clearance of HCV infection and response to peg-IFN therapy[12]. In this study we explored the role of IFNL4 polymorphisms and on-treatment change of viral load and alanine transaminase (ALT) in predicting the response to peg-IFN+RBV therapy in a cohort of patients affected by chronic HCV genotype 1-4 infection and low-stage fibrosis.

PATIENTS AND METHODS

In this retrospective study we included patients with chronic HCV infection who underwent antiviral therapy with peg-IFN alpha-2a and RBV at the Liver Unit of University of Cagliari between January 2003 and January 2012. All of them were infected by HCV genotype 1 or 4 and had a liver fibrosis stage lower than F3, as assessed by Metavir score. Chronic hepatitis C was defined as the presence of HCV RNA and abnormal ALT levels for more than six months. Clinical and laboratory data were collected at baseline and at week 2, 4, 12 and 24 of therapy. A percutaneous liver biopsy was performed before starting the antiviral therapy and liver fibrosis stage was assessed by Metavir score system. Polymorphisms from ss469415590 (IFNL4) and rs12979860 (IL28B) were analysed as follows: genomic DNA was isolated from 200 µl of serum samples with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) to test. SNPs for IFNL4were analysed in 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, USA) using an Assay On-demand and two Custom TaqMan SNP Genotyping Assays, respectively. HCV genotype was detected by Versant HCV genotype 2.0 Assay (LIpa-Siemens, Erlangen, Germany). HCV RNA was quantified by COBAS AMPLICOR/HCV Monitor (Roche, Mannheim, Germany) and real-time PCR assay (COBAS AmpliPrep/COBAS TaqMan 48-Roche). On treatment virological response was defined as rapid virological response (RVR) when HCV RNA was undetectable at week 4. Early virological response (EVR) was defined as the occurrence of negative HCV RNA (complete EVR) or reduction >2 logs of HCV RNA (partial EVR) after 12 weeks of treatment. SVR was defined as undetectable serum HCV RNA 24 weeks after the discontinuation of therapy. Patients were considered non-responders (NR) if HCV RNA remained positive during therapy and relapsers (REL) when there was A rebound in HCV RNA 24 weeks after the end of treatment. Continuous parametric variables were expressed as mean ± standard deviation (SD) and were compared using ANOVA or Student-t tests, as appropriate. Non-parametric variables were presented as median and range, and compared by Kruskal-Wallis Test. Categorical variables were compared using the chi-squared test. The percentage of ALT decrease during treatment was obtained by the following formula: ((Day0 level minus two-week level)/Day0 level). ROC analysis was used to assess the best cut-off of the decrease of ALT at week 2 and 4 of therapy. Logistic regression analysis was used to identify the predictors of SVR. We tested in the univariate analysis parameters at baseline, week 2 and week 4 of therapy and the variables with a $p<0.05$ were included in the multivariate logistic regression. SPSS software package (version 20.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Local ethic committee approved the study.

RESULTS

Baseline characteristics

One hundred twenty-four outpatients followed between January 2003 and January 2012 at the Liver Unit of University of Cagliari were included in the study. Eighty-one (65.3%) were infected by HCV genotype 1 and 43 (34.7%) by genotype 4. The mean age was 43.4 years (SD 10.3) and 89 (71.8%) were males. All patients received antiviral treatment based with peg-IFN+RBV for a median period of 47 weeks. In the 24-week follow-up after therapy, 78 patients (62.9%) achieved SVR, 21 patients (16.9 %) were REL and 25 (20.2%) were NR.

IFNL4Genotyping

The main characteristics of the patients stratified according the ss469415590 polymorphism (TT/TT, TT/DG, DG/DG) are shown in Table 1. Seventy-four (59.7%) patients were TT/DG, 30 (24.2%) TT/DG and 20 (16.1%)

Table 1. General characteristics of the study population, stratified according to IFNL4 genotype.

	Total (n=124)	TT/TT (n=30)	TT/DG (n=74)	DG/DG (n=20)	*p*-value
Male, n (%)	89 (71.8)	19 (63.3)	55 (74.3)	15 (75)	NS
Age, years, median (range)	43 (24-69)	42.5 (25-77)	43.5 (24-69)	39 (30-62)	NS
Previous therapy, n.(%)	28 (37.1)	5 (16.6)	18 (24.3)	5 (25)	NS
BMI, kg/m2, mean (SD)	24.8 (2.9)	24.8 (3.2)	24.7 (3.2)	24.7 (3.0)	NS
HCV genotype n. (%)					
- 1	81 (65.3)	21 (70)	44 (59.5)	16 (80)	NS
- 4	43 (34.7)	9 (30)	30 (40.5)	4 (20)	
IL28B,n. (%)					
- CC	30 (24.2)	30 (100)	0 (0)	0 (0)	<0.001
- CT	74 (59.7)	0 (0)	74 (100)	0 (0)	
- TT	20 (16.1)	0 (0)	0 (0)	20 (100)	
Platelets, cell/mm^3, mean (SD)	221190 (±50924)	217333 (±44641)	216973 (±51157)	240000 (±53808)	NS
AST, IU/L, median (range)	38 (16-230)	45.5 (20-230)	39 (18-105)	36 (16-69)	NS
ALT, IU/L, median (range)	66 (14-652)	75 (23-652)	63.5 (14-212)	60 (23-104)	NS
GGT, IU/L, median (range)	43 (9-292)	25.5 (10-114)	60 (9-292)	53.5 (21-137)	<0.001
HCVRNA, IU/ml, median (range)	712000 (600-13200000)	651500 (7940- 13200000)	903500 (600- 9550000)	385500 (36900-9080000)	NS
Weeks of therapy,median (range)	47 (12-71)	47 (24-59)	47 (18-71)	46.5 (12-50)	0.004
Decrease of ALT at week 2 (median)	-16.6%	-35.5%	-13.7%	-8.4%	0.003
Decrease of ALT at week 4 (median)	-37.9%	-58.5%	-38.1%	-28.1%	0.006
Decrease of ALT at week 12 (median)	-53.5%	-65.4%	-52%	-53.6%	NS
Decrease of ALT at week 24 (median)	-59.3%	-69.2%	-55.3%	-52.8%	0.038
RVR, n. (%)	-27 (21.8)	13 (43.3)	12 (16.2)	2 (10)	0.004
EVR, n. (%)	76 (61.3)	27 (90)	41 (55.4)	8 (40)	<0.001
SVR, n. (%)	78 (62.9)	29 (96.7)	39 (52.7)	10 (50)	0.001
Relapse, n. (%)	21 (16.9)	1 (3.3)	16 (21.6)	4 (20)	
Non-responder, n. (%)	25 (20.2)	0 (0)	19 (25.7)	6 (30)	

DG: delta-G. BMI: body mass index. AST: aspartate transaminases. ALT: alanine transaminases. GGT: gamma-glutamyl transaminases. NS: not significant. RVR: rapid virological response. EVR: early virological response. SVR: sustained virological response.

DG/DG. At baseline, only gamma-glutamyl transpeptidase level was significantly lower in TT/TT patients (TT/TT 25.5 IU/L, TT/DG 60 IU/L, DG/DG 53.5IU/L, $p<0.001$). RVR, EVR and SVR rate were significantly higher in TT/TT patients. The SVR rate in TT/TT was 96.7% compared to 52.7% in DG/TT and 50% in DG/DG ($p<0.001$).

ALT variation

The percentage of ALT decrease during therapy was different between the three genotypes of IFNL4. As showed in Figure 1a, after 2 weeks of treatment TT/TT patients had a median ALT decrease of 35.5%, which was significantly higher compared to genotypes TT/DG and DG/DG (13.7% and 8.4% respectively, $p=0.002$). After 4 weeks, the difference was lower but still significant ($p=0.006$) and at 12 weeks the reduction was similar between the three genotypes ($p=0.082$). The median rate of decrease was significantly higher in SVR group compared to REL and NR after 2 weeks (24.6% vs. 4.7%; $p=0.001$), after 4 weeks (44.6% vs. 23.5%; $p=0.001$), after 12 weeks (59.6% vs. 45.5%; $p=0.004$) and after 24 weeks (66.8% vs. 41.8%; $p=0.002$) (Figure 1b). The best cut-off obtained by ROC analysis was 20% at 2 weeks of therapy (AUC 0.679, sensitivity 60.3%, specificity 76.1%; $p<0.001$) and 60% at 4 weeks (AUC 0.687, sensitivity 38.5%, specificity 95.7%, $p<0.001$). As reported in Table 2, a decrease of ALT baseline level greater than 20% after 2 weeks of treatment was significantly associated with a higher rate of SVR (60.3% vs. 39.7%; $p<0.001$). Moreover patients that achieved a 60% reduction at week 4 of therapy had an SVR rate of 93.8% vs. 52.2% in patients with a lower decrease ($p<0.001$). Less patients with genotype DG/DG had a 60% reduction at week 4 (10%) compared to the other genotypes (20% in TT/DG, 50% in TT/TT, $p=0.002$). However, patients without favorable genotype, but showing ALT decrease, still achieved a higher SVR rate compared to those without ALT reduction (88% vs. 44%, $p<0.001$).

Predictors of response to treatment

In multivariate logistic regression (Table 2), factors significantly associated with SVR were TT/TT genotype ($p=0.029$, OR=10.74, 95%CI 1.28-89.84), RVR ($p=0.019$,

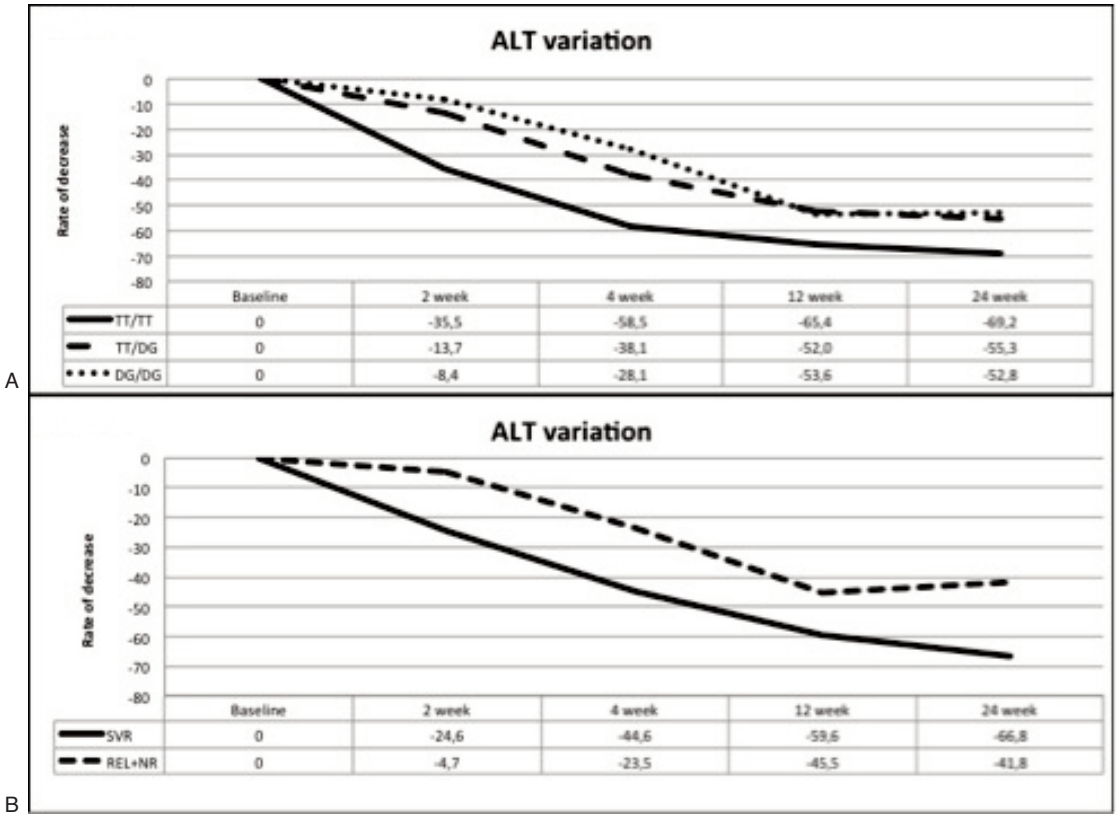

Figure 1. *A,* Rate of ALT decrease at different time points of treatment according to IFNL4 polymorphisms. *B*, Rate of ALT decrease at different time points of treatment according to virological response.

OR=6.68, 95%CI 1.36-32.87) and ALT reduction at week 4 of therapy >60% (p=0.005, OR=9.46, 95%CI 1.99-45.08). On the other hand, the absence of RVR (p=0.018, OR 6.751, 95%CI 1.38-33) and the combination of an unfavorable IFNL4 genotype (TT/DG or DG/DG) with a reduction of ALT at week 4 <60% (p<0.001, OR=0.06, 95%CI 0.02-0.23) were negative predictors of SVR. In particular, only 44.2% of patients showing this combination achieved a SVR compared to those with the same unfavorable genotype, but greater ALT decrease (88.2%, p<0.001) (Figure 2b).

DISCUSSION

The new anti-HCV drugs are highly effective, being able to cure more than 90% of treated patients[4]. However, they are very expensive and their prescription to all infected subjects is economically unsustainable for most countries worldwide. Moreover, their availability is extremely limited in many low-income countries[5]. Similarly to anti-HIV treatment[13], the possibility to identify those individuals that can be cured with the less expensive therapy (peg-IFN+RBV) would be very useful to focus the financial resources on patients that will require the new DAAs to achieve a sustained virological response. In this retrospective analysis we aimed at identifying factors that could predict the response to peg-IFN+RBV within 4 weeks of therapy. We focused on patients affected by HCV genotype 1 and 4 and low-stage fibrosis, because they represent the majority of HCV infected patients in the developing country[2]. First, we analysed the role of IFNL4 genes, a novel transcribed region recently identified by Prokunina-Olson et al[9], and located close to the three interferon lambda genes: IFNL1 (IL29), IFNL2 (IL28A) and IFNL3 (IL28B). IL28B polymorphisms are the strongest predictors of response to therapy in HCV genotype 1[7,8,14-18]. The region of IFNL4 harboured a dinucleotide variant (ss469415590) that has been found in two alternative forms, either DG or TT alleles. The one-base deletion in the DG variant results in a frameshift, which in turn produces the full-length protein, called IFNL4; on the opposite, the TT variant does not produce IFNL4[9,11]. In our cohort, an important peculiarity was that IFNL4 and IL28B polymorphisms showed a perfect match: TT/TT with CC, TT/DG with CT and DG/DG with TT. Data from literature indicates that the correspondence between IL28B and IFNL4 genotypes is about 92% in the Caucasian ethnicity[9], so it is not clear if our findings represented the real distribution of these alleles or if they were due to an artefact related to the small sample size. Therefore, from nowhere we refer only to IFNL4 genotype when commenting the results. It is noteworthy that all of patients with the TT/TT genotype cleared the virus after a peg-IFN+RBV course of treatment and only 1 of them relapsed. On the other hand, only half of the patients with the unfavourable genotype (DG/DG) or heterozygosis (TT/DG) achieved SVR. The pathophysiological mechanism that links IFNL4 polymorphisms and HCV clearance after treatment with peg-IFN+RBV has been investigated in several studies. In vitro, IFNL4 induces the expression of interferon-stimulated genes (ISG)

Table 2. Characteristics of patients stratified by treatment response and univariate and multivariate logistic analyses of predictors of sustained virological response (SVR).

	SVR	REL+NR	Univariate logistic regression			Multivariate logistic regression		
	78 (62.9%)	46 (37.1%)	OR	95% I.C.	*p* value	OR	95% I.C.	*p* value
Male, n. (%)	59 (66.3)	30 (33.7)	1.66	0.75-3.66	NS			
Age, years (median)	43	42.5	0.99	0.96-1.03	NS			
Previous therapy, n. (%)	17 (60.7)	11 (39.3)	0.89	0.37-2.11	NS			
BMI, kg/m2 (mean)	24.7	25.1	0.99	0.84-1.08	NS			
HCV genotypes n. (%)								
1	51 (63)	30 (37)	1.01	0.47-2.17	NS			
4	27 (62.8)	16 (37.2)	0.99	0.46-2.13	NS			
INFL4 genotypes (%)								
TT/TT	29 (96.7)	1 (3.3)	26.63	3.48-203.6	0.002	10.74	1.28-89.84	0.029
TT/DG	39 (52.7)	35 (47.3)	0.314	0.14-0.71	0.005	0.95	0.31-2.90	NS
DG/DG	10 (50)	10 (50)	0.53	0.20-1.39	NS			
Platelets, cells/mm^3 (mean)	216413	223346	1.00	1.00-1.00	NS			
AST, IU/L (median)	39.5	37.5	1.01	0.99-1.02	NS			
ALT, IU/L (median)	67	62	1.01	1.00-1.02	NS			
GGT, IU/L (median)	38	69.5	0.99	0.98-0.99	0.002	0.99	0.98-1.00	NS
HCVRNA, IU/ml (median)	414000	1090000	1.00	1.00-1.00	NS			
Decrease ALT after 2 weeks >20%	47 (79.6)	12 (21.4)	4.30	1.93-9.55	<0.001	2.06	0.77-5.48	NS
Decrease ALT after 4 weeks >60%	30 (93.8)	2 (6.2)	13.75	3.10-60.93	0.001	9.46	1.99-45.08	0.005
Decrease ALT after 2 weeks (median)	24.6%	4.7%	0.99	0.98-1.00	0.040	1.00	0.99-1.02	NS
Decrease ALT after 4 weeks (median)	44.6%	23.5%	0.99	0.98-1.00	NS			
RVR (%)	25 (92.6)	2 (7.4)	10.38	2.33-46.26	0.002	6.68	1.36-32.87	0.019

REL: relapser. NR: non-responder. IFNL: interferon lambda. DG: delta-G. BMI: body mass index. AST: aspartate transaminases. ALT: alanine transaminases. GGT: gamma-glutamyl transaminases. RVR: rapid virological response. EVR: early virological response. SVR: sustained virological response.

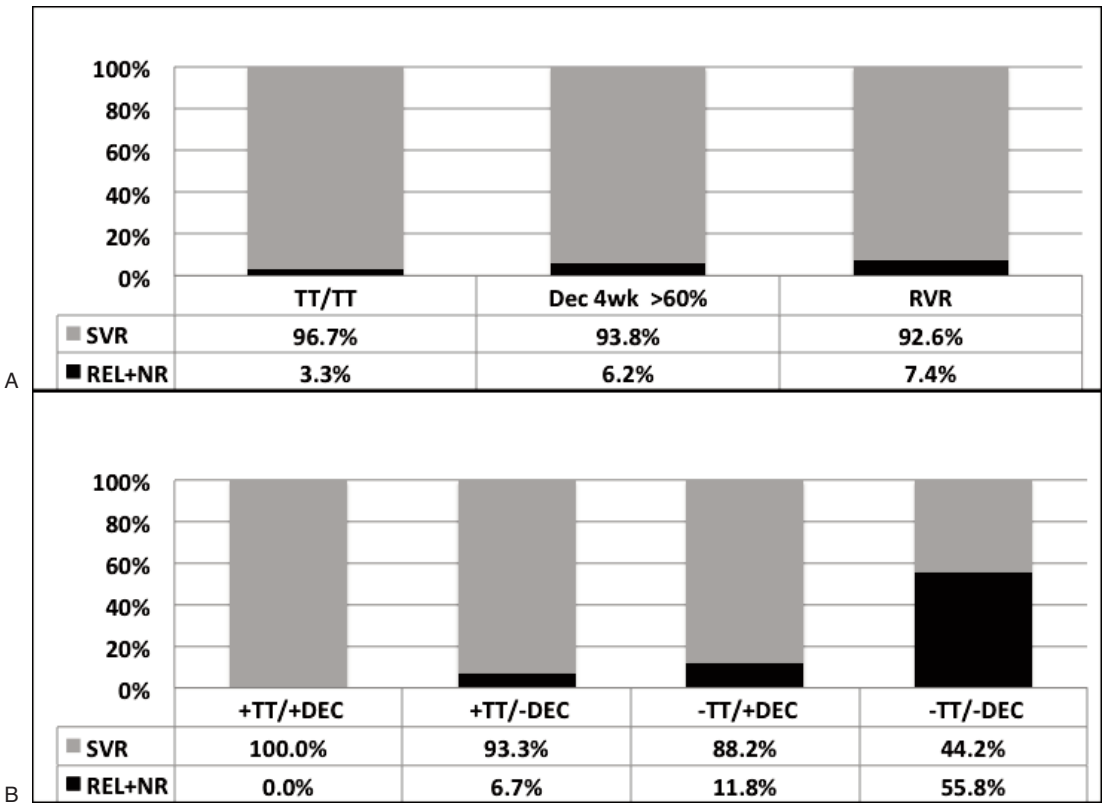

Figure 2. *A,* Percentage of SVR according to the presence of TT/TT IFNL4 genotype, decrease greater than 60% after 4 weeks of treatment and Rapid Virological Response (RVR). *B*, Percentage of SVR according to the combination of TT/TT genotype and ALT decrease greater than 60% after 4 weeks of treatment: presence of both of them (+TT/+DEC), TT/TT genotype without decrease (+TT/-DEC), decrease >60% at 4 wk in unfavourable IFNL4 genotype (-TT/+DEC) and the absence of both of them (-TT/-DEC).

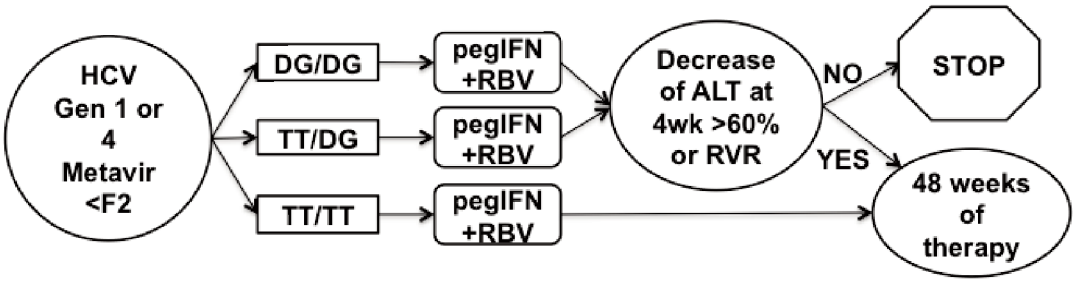

Figure 3. Treatment algorithm based on IFNL4 genotype and the presence of RVR or a ALT decrease greater than 60% after 4 weeks of therapy.

in a similar pattern to those induced by peg-IFN alpha and IL28B (9). A high baseline hepatic ISG expression correlates with poor response to peg-IFN therapy[19,20] and it is associated with the unfavourable IL28B genotype[21]. Similarly, HCV infected patients carrying the unfavourable IFNL4 genotype DG/DG, and to a lesser extent ΔG/TT heterozygosis, showed a persistent and dose-dependent expression of IFNL4 ISG in the hepatocytes[9]. Although moderate ISG expression can partially impair viral replication, it also stimulates negative regulatory pathways that ultimately reduce hepatocellular sensitivity to exogenous IFN. Therefore the IFN-alpha administered during therapy might fail to induce an effective inflammatory response able to eradicate the infection[22,23]. On the contrary, patients with the favourable IFNL4 allele TT/TT show lower level of hepatic ISG expression at baseline[9]. Although this phenomenon might result in a higher baseline viral load, hepatocytes can be more sensitive to IFN stimulation compared to the unfavourable genotype[22,23]. We have also studied the potential role of the baseline level of alanine aminotransferase (ALT) and the decrease of this enzyme during treatment as predictors of SVR. Controversial results have been reported regarding the predictive value of ALT level at baseline[24-28], while so far few studies have described ALT changes during therapy[29]. In our study, the absolute values of ALT at baseline and during treatment were not significantly associated with SVR. However, when considering the degree of variation at predefined time points during treatment, we found that patients achieving SVR had a higher decrease compare to nonresponders and relapsers, especially after 2 weeks and 4 weeks of therapy. A significant difference has also been found among the IFNL4 genotypes, because TT/TT patients presented the highest decrease of ALT at each time point of therapy. Similarly to the differences among the classes of response, the gap with the non-favourable genotypes was greater after 2 weeks and 4 weeks. However the 60% cut-off of the decrease at week 4 showed a higher specificity than the 20% cut-off at 2 weeks of treatment (95.7% vs. 76.1%). These results were confirmed in the multivariate analysis, where a decrease greater than 60% after 4 weeks of therapy was a predictor of SVR together with TT/TT genotype and RVR. The rate of SVR for each of these predictors was more than 90%. When we looked at the negative predictors, we found that patients without a favourable TT/TT genotype who presented a decrease of basal ALT less than 60% had a risk of treatment failure 13 times higher. In fact, the combination of TT/TT with the 60% decrease at week 4 had a significantly higher rate of SVR when compared to the contemporary absence of both of them. Based on these findings, we propose a stopping rule for patients carrying the unfavourable IFNL4 genotype (TT/DG or DG/DG) according to the presence of the 60% decrease of ALT after 4 weeks of therapy and/or a RVR (Figure 3). Patients who present one of these features have high chance to achieve SVR and should complete a 48-week course with peg-IFN and RBV.

CONCLUSIONS

We suggest that dual therapy could still be an effective treatment in selected subsets of patients with HCV genotype 1 or 4 infection and low-stage fibrosis. Genotyping of IFNL4 polymorphisms and the variation of ALT serum level and viral load during treatment represent affordable tools to identify ideal candidates for peg-IFN+RBV therapy. The use of our algorithm may help to reduce the number of HCV-infected patients requiring the new DAAs and therefore contribute to optimize the healthcare-related costs.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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