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Implications of Interleukins in Liver Fibrosis and Sustained Virological Response in Patients with Viral Hepatitis C

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Abstract

Introduction: Single Nucleotide Polymorphisms (SNPs) of IL 28 B and IL 10 were associated with sustained virological response (SVR) and liver fibrosis in chronic hepatitis C patients treated with peg-interferon (Peg IFN) and Ribavirin (RBV). **Methods:** We studied 188 patients with chronic hepatitis C who received antiviral therapy.
RNA HCV was determined by RT-PCR and liver fibrosis was evaluated by transient elastography (Fibroscan) and serological tests (APRI score). The IL 28 B rs12979860 and IL 10 R -1087 (rs1800896) SNPs were genotyped using Custom Taqman SNP Genotyping Assays. **Results:** From 176 patients, 98 (55.68%) had SVR, 59 (33.52%) were non-responders and 19 (10.80%) were relapsers. SVR was strongly associated with IL 28 B SNP genotypes (p<0.0005). Advanced/severe fibrosis was observed in 109 cases (57.97%). Fibrosis was not associated with IL 28 B SNP. IL 10 R SNP was a predictor for liver fibrosis: severe fibrosis was present in 66.67% of patients with GG genotype, 26.98% with GA and 51.02% with AA genotypes. The G allele of IL-10 SNP was associated with advanced fibrosis (OR: 2.40, p = 0.018). **Conclusions:** The determination of the interleukins polymorphisms is important in predicting the viral response to interferon therapy and evolution of liver fibrosis.

Keywords: interleukins, liver fibrosis, hepatitis C

Rezumat

Introducere: Polimorfismele unui singur nucleotid (SNP) a IL 28 B și IL 10 au fost asociate cu un răspuns viral susținut (RVS) și fibroza hepatică la pacienții cu hepatită cronică C tratați cu interferon peg-(Peg IFN) și ribavirină (RBV). **Metode:** Am studiat 188 de pacienți cu hepatită cronică C, care au primit terapie antivirală.
ARN HCV a fost determinat prin RT-PCR și fibroza hepatică a fost evaluată prin elastografie tranzitorie (Fibroscan®) și teste serologice (scor APRI). SNP IL 28 B rs12979860 și IL 10 R -1087 (rs1800896) s-au genotipat folosind Custom Taqman SNP Genotyping Assays. **Rezultate:** Din 176 pacienți, 98 (55.68%) au prezentat RVS, 59 (33,52%) au fost non-responderi și 19 (10,80%) cu recădere. RVS s-a asociat cu genotipurile SNP IL 28 B (p <0.0005). Fibroza avansată a fost observată la 109 cazuri (57,97%). Fibroza nu a fost asociată cu IL 28 B SNP. IL 10 R SNP a fost predictor pentru fibroza hepatică: 66,67% din pacienți au prezentat genotip GG, 26.98% genotip GA și 51.02%, genotip AA. Alela G IL-10 SNP s-a asociat cu fibroza avansată (OR: 2,40, p = 0,018). **Concluzii:** Determinarea polimorfismelor interleukinelor este importantă în prezicerea răspunsului viral la terapia cu interferon și evoluția fibrozei hepatice.

Cuvinte cheie: interleukine, fibroza hepatică, hepatita C

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of progressive hepatic disease with a high impact on human health worldwide. Patients with chronic hepatitis C have an increased risk of complications with need of hospitalization for different associated pathology. Liver fibrosis is correlated with high morbidity and mortality[1]. Liver cirrhosis is frequently associated with anemia caused by gastrointestinal hemorrhage from esophageal varices or from angiodysplasia of the antrum[2,3].

Recent studies estimate that more than 185 million people worldwide have been infected with HCV, of which 350,000 die each year[3,4]. In Romania the prevalence is relatively high (2.3%) representing 425.000 infected individuals, predominantly with the genotype 1b[5]. Nearly 80% of HCV patients develop chronic liver disease determined by the ability of the virus to evade the host immune mechanisms[6]. The impact of the viral infection on the liver tissue range from histological lesions of minimal to intense fibrosis, evolution to cirrhosis and hepatocellular carcinoma. Cirrhosis due to hepatitis C infection is currently the leading cause of liver transplantation[7,8].

In HCV infection a pivotal role is played by the host immune response with cytokines as main mechanisms involved in host defense that induce an inflammatory response, which leads to liver injury, and they also have antiviral effects. The cytokines synthesis is influenced by genetic factors[9,10], like single nucleotid polymorphisms (SNPs). The inappropriate cytokine secretion can influence the outcome of HCV infection[11].

The classical therapy of HCV infection is peg-interferon (Peg-IFN) associated with Ribavirin (RBV). The interferon treatment outcome can be influenced by the level of host cytokines[12].

The modern therapies of hepatitis C involve protease inhibitors (PIs), direct acting antivirals (DAA). This agents are used in various combinations, with or without Ribavirin, with a success rate of 95-100% in patients infected with HCV genotype 1[3].

In limited resources countries, we need to prioritize the patients who will receive DAA therapies, due to the high cost. In Romania it was recently approved a therapeutic protocol based on Ombitasvir, Paritaprevir, Ritonavir and Dasabuvir. Currently, these are reserved to patients who fail to achieve sustained virological response (SVR) after interferon therapy, those with severe fibrosis/cirrhosis (F4), patients who relapse after liver transplantation, those with genotype 1 HCV with advanced fibrosis (F3) and patients with contraindications to interferon therapy.

The interleukin 28 B (IL 28 B) (interferonλ-3) activates the JAK-STAT pathway, which has antiviral activity and is involved in natural HCV clearance[13,14]. Many studies showed that SNPs around the IL 28 B gene are associated with response to Peg-IFN and RBV therapy. Ge et al[15] identified a SNP (rs12979860) 3 kilobases upstream from the IL 28 B gene associated with sustained viral response (SVR) to interferon therapy.

Interleukin-10 (IL 10), is produced by macrophages, monocytes, and T cells. It inhibits the function of cytotoxic CD8 + T, NK and other antigen-presenting cells, the activation of CD4+ T-helper cells and also the synthesis of collagen by the hepatic stellate cells. Therefore, IL 10 is a suppressor of the immune reaction and inhibits inflammatory responses by downregulating the production of pro-inflammatory cytokines. There is a lot of variation of IL 10 levels between individuals that probably due to variations in the promoter of IL 10 gene, including genetic polymorphisms. IL 10 can downregulate type-1 helper T cell (Th1) cytokines, like IL 1, IL 6, TNF-α and IFN-γ[16].

These cytokines are correlated with increasing of necroinflammatory activity and with histological fibrosis in chronic infection with HCV[17]. The progression of liver disease is greatly influenced by the imbalance between Th1 and Th2 cytokines[18]. Other studies suggest that IL 10 has also a modulatory effect on liver fibrogenesis[19].

Some polymorphisms of IL 10 gene influence the secretion level, which has effects on the liver inflammation and progression to fibrosis and also can be associated with spontaneous viral cleareance[18,20,21].

AIM

Our study aims to evaluate the predictive value of IL 28 B and IL 10 gene polymorphisms in progression of liver fibrosis and interferon treatment response in genotype 1 HCV-infected patients.

METHODS

Ethics

This research has been carried out in accordance with the guidelines of the 1975 Declaration of Helsinki and has been approved by both the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania.

For the enrolled patients from Portugal we have the agreement of Ethics Committee of Hospital Santa

Maria from Lisbon, Portugal. The patients provided a written informed consent of participation in the study.

Study design

The study enrolled a number of 188 patients with chronic hepatitis C, 133 admitted in Medical Clinics no. 1 and 2, Emergency County Hospital Craiova and 55 patients from Santa Maria Hospital, Lisbon, Portugal. From these patients only 176 completely followed the RNA monitoring protocol. All the patients received 180 μg subcutaneously of peg-interferon and Ribavirin for 48 weeks, and were followed for 24 weeks after the end of treatment.

Laboratory assessment

The chronic hepatitis C was diagnosed based on elevation of liver aminotransferases (ALT, AST) which persisted more than 6 months and the presence of anti-HCV determined by ELISA. The diagnosis was confirmed by detection of HCV RNA.

We assessed every three months the biochemical markers: AST, ALT, gamma glutamil peptidase (GGT), alkaline phosphatase (ALP), glucose, urea, serum creatinine. We also performed full blood count (FBC) and coagulation tests (INR, APTT).

The HCV RNA load was assessed at 0, 12, 24, 48 and 72 weeks of treatment by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Quantitation of HCV RNA in the plasma and HCV genotyping was carried out using Cobas 6800 (Roche Molecular Diagnostics).

Liver fibrosis was evaluated in all 188 patients before antiviral treatment by transitory liver elastography (Fibroscan®) and serological tests (APRI score).

FibroScan® is the most currently used physical method for assessing fibrosis in patients with chronic viral hepatitis C. It is a noninvasive method that measures liver tissue elasticity based on unidimensional transient liver elastography. The result is given in kilopascals (kPa) and can vary between 2.5 and 75 kPa. We classified the patients as follows: without liver fibrosis (below 6.0 kPa), with moderate/mild fibrosis (6-9 kPa) and with advanced/severe fibrosis or cirrhosis (>12 kPa). The cut-off used was 7.1 kPa for moderate fibrosis and 12.5 kPa for cirrhosis[22-24].

The APRI (AST-to-Platelet Ratio Index) score is based upon the degree of fibrosis, portal pressure, secretion of trombopoetin, retention of platelets by the spline and the AST clearance. We calculated APRI score as the ratio between AST / (upper limit of normal range of platelet count / platelet count of the patient (10^9/L)) × 100[24].

Genotyping of the interleukins' SNPs

Blood samples have been collected on EDTA and genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, USA) following the manufacturer's protocol.

All the polymorphisms were genotyped by allelic discrimination polymerase chain reaction assays (5' nuclease assay) using predesigned TagMan SNP Genotyping Assays (Applied Biosystems, USA): IL 28 B rs12979860 and IL 10 R rs1800896. For this study we used a two steps cycling protocol with a first at 95°C for 10 min step, followed by a two phase second step of 50 cycles with the first at 95°C for 15s and the second one at 60°C for 1 min.

We genotyped only 133 patients for IL 10 R SNP.

Statistical analysis

Differences in frequency between groups were analyzed using *chi*-square, or in case of small values of expected frequencies (as in most cases), Fisher's exact test. The differences of frequencies of cases among various groups was expressed as odds ratios, as well as the coefficients of the multivariate analyses. It is important to mention we had a cohort, non-randomized study design. For most analysis, we included the relapsers in the non-responder category. For quantitative variables we used the Student *t* test and the univariate ANOVA analysis to evaluate the significance of the differences between groups. Effect sizes were measured by Cramer's V for *chi* square tests, Cohen's d for Student *t* tests. Statistical calculations were performed using SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

From the 188 patients, 123 were women (65.43%) and 65 men (34.57%), 127 patients (67.55%) were aged over 50 years. A total of 136 patients (72.73%) came from urban areas.

Following evaluation by Fibroscan® 109 patients (57.98%) had advanced/severe fibrosis (F3-F4) and 79 (42.02%) had no fibrosis or mild/moderate fibrosis (F0-F2).

Anemia prevalence in the studied group was 30.85%, (mean hemoglobin 13.39 g/dL), and thrombocytopenia prevalence was 41.71% (average platelet count was 189,000 media/mL). White blood cells count was normal, with an average of 5,598 cells/mL.

Hepatic steatosis was present in 41 patients (21.81%), peripheral neuropathy in 16 patients (8.51%), hypothy-

roidism in 9 patients (4.79%) and diabetes in 24 patients (12.77%).

Viral load had an average of 2.668.385 IU/mL, 118 patients (62.77%) had a load over 400.000 IU/mL. Biochemical parameters of liver function showed cytolysis. Thus, ALT had an average of 76.39 IU/L, AST 94.12 IU/L, GGT 98.64 IU/L and ALP 84.50 IU/L. The kidney function was relatively preserved as blood urea had an average value of 38.90 g/L and creatinine 0.84 g/L.

Analysis of viral response (Table 1) revealed that from 176 patients who completely followed the RNA monitoring protocol, 98 (55.68%) achieved sustained virological response, 59 (33.52%) were non-responders and 19 (10.80%) relapsers. We observed a great reduction of HCV-RNA in weeks 4 and 12 of therapy in all patients. We also observed a marked HCV RNA decrease in responders in the first 12 weeks of therapy, this being a positive prognostic factor for SVR.

By comparing clinical and biological parameters among responders and non-responders (Table 2) we observed statistically significant differences. Positive predictive factors were age under 50 years (p=0.020), mild liver fibrosis (F1) (OR:6.91, p=0.038), viral load under 400,000 UI/mL before treatment (OR: 2.21, p=0.014). Negative predictive factors included advanced/severe fibrosis (F3-F4) (OR:0.42, p=0.013), high AST (p=0.003), ALT (p=0.006) and GGT (129.95 UI/L in non-responders vs. 70.67 UI/L in responders, p=0.001) levels.

Hemoglobin levels were normal before treatment in responders (13.35 g/dL) and non-responders (13.47 g/dL) and decreased during antiviral therapy and recovered afterwards. The number of thrombocytes decreased in the first week of treatment, and recovered to normal values at the end of therapy in responders, but remained decreased in non-responders. By analyzing the associated pathology, we observed that patients with liver steatosis had a lower rate of SVR.

There was a strong link between IL 28 B SNP rs12979860 genotypes and response to antiviral therapy (Table 3). Patients with the CC genotype who received the combination therapy of Peg-IFN and RBV had higher SVR rates (78.95%, OR: 4.56, 95% confidence interval: 2.152 to 9.666, p <0.001) than subjects who had CT (50.53%) or TT genotypes (23.08%) (OR: 0.17, 95% confidence interval: 0.062 to 0.471, p = 0.0003).

The response rate to interferon therapy was higher in those with IL 10 R SNP GA (77.77%) and AA (59.18%) genotypes (Table 4), compared to those with GG genotype (47.61%) (OR: 2.200, 95% confidence interval: 0,701- 6.889, p = 0.1251). We consider that the G allele could be a negative predictive factor for SVR, but it did not reach the statistical significance because the lot contained only 17 responders with the homozygote GG genotype.

Table 1. Baseline characteristics of patients with and without sustained virological response to Peg-IFN + RBV combination therapy

Parameter	Responders (n=98)	Non-responders and relapsers (n=78)	Test statistic (OR / Student's t)	p
Females Males	60(61.22%) 38 (38.78%)	53(67.95%) 25(32.05%)	1.342 (0.721-2.500)	0.355
Age <=50 years **Age > 50 years**	**43 (39.79%)** **55 (56.10%)**	**21 (26.92%)** **57(73.08%)**	**2.160** **(1.128-4.136)**	**0.020**
Fibroscan® stage				
F0	10 (10.20%)	8 (10.26%)	1.002	0.995
F1	**8 (8.16%)**	**1 (1.28%)**	**6.909**	**0.038**
F2	30 (30.61%)	16 (20.51%)	1.702	0.143
F3	30 (30.61%)	30 (38.46%)	0.972	0.928
F4	20 (20.41%)	23 (29.49%)	0.618	0.172
Mild/moderate fibrosis (F1-F2)	**38 (43.19%)**	**17 (24.28%)**	**0.42**	**0.013**
Severe/advanced fibrosis (F3-F4)	**50 (56.81%)**	**53 (75.72%)**		
APRI score	0.66+/-0.42	1.26+/-1.47	0.136	0.195
Steatosis present Steatosis absent	20 (20.41%) 78 (79.59%)	19 (24.36%) 59 (75.64%)	0.796	0.531

APRI = AST to Platelet Ratio Index

Table 2. Laboratory parameters of patients with and without sustained virological response to Peg-IFN + RBV combination therapy

Parameter	Responders (n=98)	Non-responders and relapsers (n=78)	Test statistic (OR/ Student's t)	p
HCV RNA <400,000 UI/mL **HCV RNA >= 400,000 UI/mL**	**43 (43.87%)** **55 (56.13%)**	**21 (26.92%)** **57 (73.08%)**	**2.211 (1.171-4.173)**	**0.014**
AST (UI/L)	**63.05+/-50.86**	**91.77+/-65.97**	**0.233**	**0.003**
ALT (UI/L)	**81.84+/-65.43**	**110.41+/-73.65**	**0.225**	**0.006**
GGT (UI/L)	**70.67+/-80.88**	**129.95+/-271.79**	**0.128**	**0.001**
ALP (UI/L)	90.07+/-52.53	78.17+/-31.44	0.040	0.836
Glucose (g/L)	94.82+/-21.37	101.78+/-16.98	0.259	0.079
Urea (g/L)	34.65+/-11.27	43.57+/-16.27	0.274	0.3056
Creatinine (g/L)	0.90+/-0.34	0.78+/-0.12	0.103	0.621
Hemoglobin (g/dL)	13.35+/-1.20	13.54+/-1.41	0.118	0.162
White blood cells (no./mL)	5569+/-2479	5531+/-2701	0.009	0.942
Thrombocytes < 150.000/mL Thrombocytes >= 150.000/mL	32 (32.65%) 66 (67.35%)	37 (47.44%) 41 (52.56%)	0.774	0.152
INR	0.95+/-0.01	0.94+/-0.16	0.098	0.769

HCV = Hepatitis C Virus, RNA = Ribonucleic Acid, AST = Aspartat AminoTransferase, ALT = Alanin Transaminase, ALP = Alkaline Phosphatase, INR = International Normalized Ratio

Table 3. The rate of SVR in correlation with the IL 28 B SNP genotypes

IL 28 B SNP	CC	CT	TT	Total
non-responders	12 (21.05%)	46 (49.47%)	20 (76.92%)	78 (44.32%)
responders	45 (78.95%)	47 (50.53%)	6 (23.08%)	98 (55.68%)
Total	57 (100%)	93 (100%)	26 (100%)	176 (100.00%)

Table 4. The rate of SVR in correlation with the IL 10 R SNP genotypes

IL 10 R SNP	AA	GA	GG	Total
non-responders	20 (40.82%)	23 (22.23%)	11 (52.39%)	54 (%)
responders	29 (59.18%)	40 (77.77%)	10 (47.61%)	79 (%)
Total	49 (100%)	63 (100%)	21 (100%)	133 (100%)

We observed marginally significant differences between patients with fibrosis F0-F2 and F3-F4 regarding the following parameters (Table 5): patient age over 50 years (62.03% vs. 70.67%, OR: 1.47, p=0.0507), sustained virological response to treatment (65.75% vs. 48.54%, OR: 0.49, p=0.0236), baseline HCV RNA (4.079.865 vs. 1.722.831 IU/L, Student's *t:* 2.00, p=0.0471) (Table 6), serum glucose (93.69 versus 102.96 g/L). AST had higher values in patients with advanced/severe fibrosis (85.18 IU/L) than in those with mild/moderate fibrosis (64.31 IU/L) (p=0.0235). Thrombocytopenia under 150.000/mL was a positive predictor of advanced/severe fibrosis. A total of 56 patients (70.89%) from those with mild/moderate fibrosis had platelet counts above 150,000/mL (Table 7), compared with only 61 (55.96%) patients with advanced/severe fibrosis. APRI score has differentiated patients with mild/moderate fibrosis (average APRI: 0.72) from those with advanced/severe fibrosis (average APRI: 1.28).

We examined the ability of APRI score to differentiate between mild and advanced liver fibrosis. For this we classified patients according to the Fibroscan® Score. As shown in Table 5, APRI score is higher in patients with advanced fibrosis. We tried to set a cut-off of APRI score for obtaining an appropriate sensitivity and specificity for differentiating between patients with mild/moderate fibrosis and those with advanced/severe fibrosis.

We used a classification of liver fibrosis as mild/moderate (F0-F3) and advanced fibrosis/cirrhosis (F4). Using this classification, we chose a cut-off value of 0.5

Table 5. Baseline characteristics of patients with mild/moderate fibrosis and with advanced/severe fibrosis

Parameter	Fibrosis F0-F2 (n=79)	Fibrosis F3-F4 (n=109)	Test statistic (OR/Student t)	Cramer V, Cohen's d	p
Females Males	53 (67.09%) 26 (32.91%)	69 (63.30%) 40 (36.70%)	1.17	0.0375	0.6830
Age (years)	51.40+/-9.06	55.86+/-9.82	-2.78	-0.46	0.9969
Age <=50 years Age > 50 years	**30 (37.97%)** **49 (62.03%)**	**32 (29.36%)** **77 (70.67%)**	1.47	0.19	0.0507
Responders Non-responders	**51 (65,75%)** **28 (34,25%)**	**53 (48,54%)** **56 (51,46%)**	0.52	-0.17	0.0301

Table 6. Laboratory parameters of patients with mild/moderate fibrosis and with advanced/severe fibrosis

Parameter	Fibrosis F0-F2 (n=79)	Fibrosis F3-F4 (n=109)	Test statistic (OR/Student t)	Cramer V, Cohen's d	p
HCV RNA (UI/L)	**4.079.865+/-11.400.000**	**1.722.831+/-2.871.815**	**2.00**	**0.31**	**0.0471**
HCV RNA <=400.000 UI/mL HCV RNA > 400.000 UI/mL	26 (32.91%) 53 (67.09%)	43 (339.45%) 66 (60.55%)	1,18	0,04	0,586
AST (UI/L)	**64.31+/-55.11**	**85.18+/-61.53**	**-2.29**	**-0.35**	**0.0235**
ALT (UI/L)	88.84+/-76.70	97.69+/-64.81	-0.78	-0.12	0.4382
GGT (UI/L)	110.0+/-278.54	88.71+/-17.47	0.36	0.11	0.7234
ALP (UI/L)	92.0+/-47.79	79.65+/-38.66	0.75	0.29	0.4584
Glucose (g/L)	**93.69+/-14.04**	**102.96+/-21.91**	**-1.79**	**-0.51**	**0.0791**
Urea (g/L)	41.25+/-16.12	35.37+/-11.24	0.81	0.41	0.4343
Creatinine(g/L)	0.81+/-0.17	0.87+/-0.32	-0.66	-0.27	0.5144
APRI score	**0.72+/-0.63**	**1.28+/-1.49**	**-2.41**	**-0.50**	**0.0177**

HCV = Hepatitis C Virus, RNA = Ribonucleic Acid, AST = Aspartat AminoTransferase, ALT = Alanin Transaminase, ALP = Alkaline Phosphatase, INR = International Normalized Ratio, APRI = AST to Platelet Ratio Index

for APRI score, which yielded a sensitivity of 83.33% and a specificity of 53.33%, useful for selecting patients for further analysis of liver fibrosis. The area under the ROC curve (*Receiver Operating Characteristic*) (AUC) was 0.7541 (Figure 1).

We also investigated the capacity of AST/ALT ratio (AAR) to differentiate patients with advanced/severe fibrosis, with a cut-off of 0.50 had a sensitivity of 65% and a specificity of 49% (AUC=0.5855).

There are no significant differences between IL 28 B genotype distributions in patients with F0-F2 fibrosis and F3-F4, as the Chi square test p=0.808, >0.05 (Table 8).

IL 28 B genotypes were not significantly associated with the degree of liver fibrosis assessed by transient dimensional elastography. The frequencies of genotypes were similar in patients with advanced/severe fibrosis and mild/moderate fibrosis (Table 8). Severe fibrosis was observed in 56.90% of patients with the CC genotype, 59.57% in CT and 54.17% in TT (p=0.8660).

IL 10 R SNP genotypes were in Hardy-Weinberg equilibrium (p=0.3528). Advanced/severe fibrosis (F3-F4) was present in 51.02% of patients with genotype AA, 26.98% in those with genotype GA and 66.67% in patients with genotype GG (Table 9).

The IL 10 R SNP G allele was more frequent in patients with severe/advanced fibrosis (OR: 2.400, 95% CI: 1.159 to 4.974, p = 0.018) and GG genotype didn't reach statistical significance as risk factor for advanced/severe fibrosis (OR: 1.155, 95% CI: 0.441 to 3.010, p = 0.7744). To note that there were only 21 patients with GG genotype, so the statistical difference didn't reach the significance level. The A allele of IL 10 R SNP was more frequent in patients with mild to moderate fibrosis (OR: 3.33, p=0.013).

We conclude that SNP -1082 of IL 10 R was a better predictor for liver fibrosis compared with IL 28 B, who had almost no predictive value.

The combination between CC genotype of IL 28 B had a protective effect regarding liver fibrosis when combined with the A allele of IL 10 R (OR: 0.698) and with the AA genotype (Table 10), (OR: 0.526), but the association didn't reached the statistical significance (p=0.2519). The GG genotype increased the risk

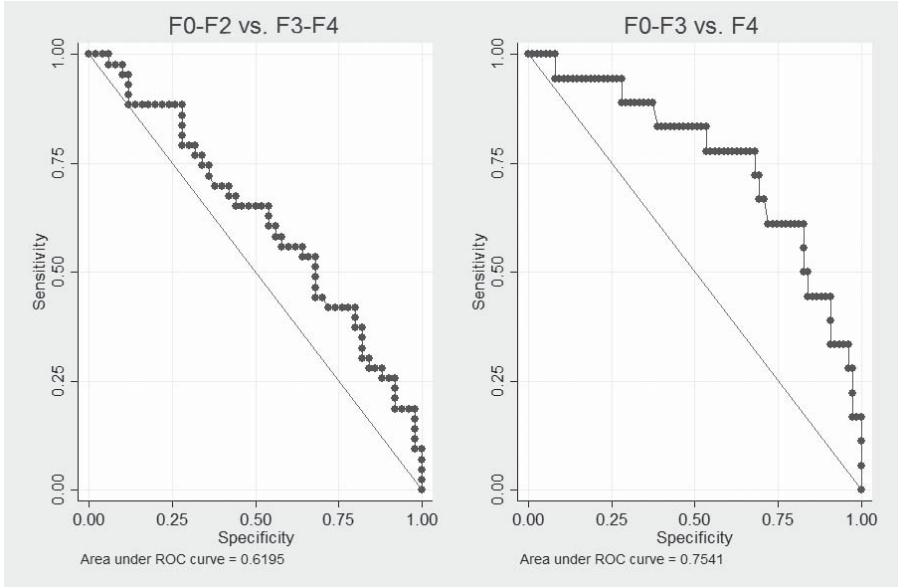

Figure 1. ROC curves for the staging of liver fibrosis using the APRI score.

of liver fibrosis, both in combination with the C allele of the IL 28 B (OR: 1,348) and with the T allele (OR: 1.091).

In conclusion the advanced/severe fibrosis was significantly associated with the presence of the G allele of IL 10 R SNP regardless of the IL 28 B SNP genotype.

We identified by multivariate logistic analysis, the following positive predictors for the odds of SVR: mild/moderate fibrosis (OR: 2.84, p=0.0897), viral load <400,000 IU/L (OR: 3.39, p=0.0169), the CC genotype (OR=7.62, p=0.0827) and the CT genotypes (OR=7.77, p=0.0221) of IL 28 B SNP. Patient age below 50 years was a positive predictor, but without statistical significance (OR: 2.26; p = 0.1117).

For **advanced/severe fibrosis** we identified the following positive predictors: platelet count <150,000/mL (OR: 15.81; p = 0.0075), the GA (OR = 26.84; p = 0.0482) genotype of IL 10). Male gender was a positive predictor, but without reaching statistical significance (OR: 5.19, p=0.1622). Age under 50 was a protective factor for advanced/severe fibrosis (OR = 0.07, p = 0.0329).

Table 7. Hematological parameters of patients with mild/moderate fibrosis and with advanced/severe fibrosis

Parameter	Fibrosis F0-F2 (n=79)	Fibrosis F3-F4 (n=109)	Test statistic (OR/ Student t)	Cramer V, Cohen's d	p
Hemoglobin (g/dL)	13.49+/-1.25	13.34+/-1.28	0.69	0.11	0.4898
White blood cells (nr/mL)	5.783+/-2.434	5.366+/-2.761	0.64	0.16	0.5273
Thrombocytes (nr./mL)	197.162+/-52.005	180.117+/-73.159	1.13	0.27	0.2588
Thrombocytes > 150.000/mL **Thrombocytes <= 150.000/mL**	**56 (70.89%)** **23 (29.11%)**	**61 (55.96%)** **48 (44.04%)**	**3.0537**	**0.81**	**0.0032**
INR	1.00+/-0.15	0.89+/-0.13	1.21	0.81	0.2669

INR = International Normalized Ratio

Table 8. The correlation between fibrosis stage and IL 28 B SNP genotypes

IL 28 B	CC	CT	TT	Total
F0-F2	25 (43.10%)	38 (40.43%)	11 (45.83%)	74 (42.05%)
F3-F4	33 (56.90%)	56 (59.57%)	13 (54.17%)	102 (57.95%)
Total	58 (100%)	94 (100%)	24 (100%)	176 (100%)

Table 9. The correlation between fibrosis stage and IL 10 R SNP genotypes

IL 10 R	AA	GA	GG	Total
F0-F2	24 (49.98%)	46 (73.02%)	7 (33.33%)	48 (36.09%)
F3-F4	25 (51.02%)	17 (26.98%)	14 (66.67%)	85 (63.91%)
Total	49 (100%)	63 (100%)	21 (100%)	133 (100.00%)

Table 11. Prevalence of the genotype and allele combinations of IL 28 B and IL 10 R SNPs in patients with mild/moderate and advanced/severe fibrosis

Combinations of genotypes and alleles	Fibrosis F0-F2 (n=85)	Fibrosis F3-F4 (n=48)	Odds Ratio (OR), 95% CI, p
IL 28 B CC + IL 10 R A allele	**22 (25.88%)**	**16 (33.33%)**	**0.698 (0.325-1.500) p=0.3610**
IL 28 B T allele + IL 10 R GG	8 (9.42%)	4 (8.33%)	1.091 (0.327-3.605) p=0.8921
IL 28 B C allele + IL 10 R GG	12 (14.12%)	5 (10.42%)	1.348 (0.459-3.926) p=0.5975
IL 28 B T allele + IL 10 R A allele	49 (57.65%)	25 (52.08%)	1.252 (0.618-2.539) p=0.5351
IL 28 B C allele + IL 10 R A allele	62 (72.94%)	36 (75.00%)	0.899 (0.404-2.001) p=0.7957
IL 28 B C allele + IL 10 R G allele	**56 (65.88%)**	**19 (39.58%)**	**2,744 (1.317-5.714), p=0.066**
IL 28 B T allele + IL 10 R G allele	**39 (45.88%)**	**12 (25.00%)**	**2.402 (1.105-5,209) p=0.0266**

DISCUSSION

The development of chronic hepatitis is highly variable among individuals due to genetic factors, the differences in viral morphology and environmental factors. The immune response has also been found to influence the outcome of HCV infection and to be related with the progression of liver fibrosis and liver cirrhosis susceptibility[25,26].

Different studies have shown that IL 28 B SNP at the polymorphic site rs129798060 and IL 10 receptor SNP at –1082 genotype are associated with evolution to chronic hepatitis C virus infection and may also influence the response to combined antiviral therapy. These associations presumably are related with the ability of IL 28 B to induce the antiviral state[11].

The protective effect of IL 28 B against HCV infection may be associated with an elevated production of IL 28 B (IFNλ-3) which has a genetic determination by polymorphisms of SNP IL 28 B[14]. This influence of IL 28 B polymorphism on SVR is also demonstrated on transplanted patients with HCV infection treated with Peg IFN and Ribavirin[27,28].

In our study we also observed a strong correlation between IL 28 B SNP rs12979860 genotypes and SVR. The highest rate of SVR was in patients with genotype CC (78.95%) compared with genotype CT (50.53%) or TT (23.08%).

IL 10 is a immunoregulatory cytokine which influence many aspects of the immune response. It could be considered a suppressor of immunity reactions because it inhibits the secretion of pro-inflammatory and antiviral cytokines like interferon-gamma (IFN-Gama) and tumor necrosis factor-alpha (TNF-a) (29). The -1082 GG genotype can produce twofold greater quantities of IL 10 compared to the AA or GA genotypes, which can interfere with host immune response[30].

The SVR rate was 47.61% in patients with GG genotype, 77.77% in those with GA genotype and 59.18% for the AA genotype. HCV patients with A allele (non-GG genotype) achieved SVR in 64.36%. This result confirms that G allele may be associated with weak therapy response.

A study on an Est-European cohort of 748 patients infected with HCV genotype 1 had found the IL 10 R GG genotype more frequent in responders (32%) compared with AA genotype (17%)[11]. Also, the combination of IL 28 B CC genotype and the A allele of IL 10 R SNP was positively associated with the SVR (OR:1.848, 95% CI 1.070-3.190, p = 0.026). We can confirm that the combination of IL 28 B CC genotype plus IL 10 R A allele was much higher in the respon-

ders (34.67%) than in non-responders (14.8%) (OR: 3.0032, p=0.017).

In a study performing a multivariate analysis on treatment response in HCV infected patients, the SVR was predicted by mild to moderate fibrosis (F0-F2), IL 28 B genotype CC and a low RNA VHC before treatment (44.44% of patients with SVR had a baseline RNA VHC <400,000 UI/mL)[31]. This fact is also sustained by other studies which combine baseline factors in order to classify patients who have a better chance to be cured with double therapy[25].

In our population of HCV infected patients, we also observed that a rapid decline in HCV RNA and negative at week 12 was a positive predictive factor of treatment response.

In our study the patients with IL 28 B SNP CC genotype had higher SVR rates (78.95%) than those with CT (50.53%) or TT (23.08%), results that confirm the previous studies.

A study on a population of 1014 Caucasian patients, observed that factors involved in SVR were young age, low viral load and absence of cirrhosis, GGT and ALP, ALT, AST levels[32]. A value of GGT under 85 IU / mL positively influenced the evolution (OR: 3.301, 95% CI: 0192-0471, P <0.001). This supports our results where we can see that those who had SVR showed average values of GGT 70.67 IU / L compared with non-responders which had 129.95 IU / L. The values of ALP were not significantly different between responders and non-responders. The average values recorded at baseline in responders for AST was 63.05 IU / L and for ALT was 81.84 IU / L were lower compared to non-responders who had intense hepatic cytolysis with AST values 91.77 IU / L and ALT 110.41 IU / L.

Liver fibrosis is one of the most important factors that influence the outcome of treatment. It is one of the main prognostic risk factors in developing cirrhosis and liver-related complications in non-viral or viral chronic hepatitis[33,34]. This may influence the treatment outcome and it is considered as a pivotal factor in taking the decision to initiate therapy. As the fibrosis progresses to more advanced stages, and finally to cirrhosis, treatment is more difficult due to many complications. The patients with F3-F4 are candidates to immediate DAA therapy.

Patients with a moderate fibrosis respond better to antiviral therapy than those with advanced grade of fibrosis or cirrhosis. This fact could be explained by a higher rate of adverse effects that appear in patients with advanced fibrosis/ cirrhosis or maybe a lower rate of infected hepatocytes in patients with mild fibrosis and SVR[35-37].

In our study mild liver fibrosis (F1) was a positive predictive factor of SVR (OR:6.91, p=0.038). Severe/ advanced fibrosis (F3-F4) was associated with a lower rate of SVR (OR: 0.42, p=0.013).

The degree of liver fibrosis is greatly dependent on the host immune response, which is correlated with interleukin polymorphisms'. Therefore, levels of circulating interleukins can influence the course of liver fibrosis. As genetic polymorphism influence the interleukins circulating levels, we investigated the relation between IL 28 B and IL 10 R polymorphism and liver fibrosis. In our study the IL 28 B SNP does not influence the degree of liver stiffness.

A study on a Chinese population of 192 patients with liver cirrhosis of various etiologies (viral hepatitis B, C and alcohol) and 192 controls genotyped the IL 10 R SNPs -592 A/C, IL 10 -819 C/T and IL 10 R -1082 A/G. The study aimed to define the genetic risk of liver cirrhosis. The multivariate regression analysis showed that individuals with the G allele of IL 10 R -1082 had a significantly higher risk for liver cirrhosis (OR: 2,14, 95% CI (0.97-1.68), p=0.050)[19].

In our HCV patients with AA genotype, the advanced/severe fibrosis (F3-F4) was present in 51.02% of patients, compared with 26.98% of patients with genotype GA and 66.67% in patients with genotype GG. The IL 10 R SNP G allele (OR: 2.40, 95% CI: 1.159 to 4.974, p=0.018) and the GG genotype (OR: 1.15) were more frequent in patients with F3/F4 fibrosis. The A allele of IL 10 R SNP was more frequent in patients with mild to moderate fibrosis (OR: 3.33, p=0.013). In this case, our results confirm that G allele was a predictive factor for developing liver fibrosis.

There is an increasing interest to find a non-invasive marker that is cost-effective for assessment of liver fibrosis and that is easy to perform and valid over a wide patient population[38].

Serum biomarkers like APRI (AST to Platelet Ratio), have practical advantages to measure fibrosis[39]. APRI score is a simple test that can reliably differentiate mild from significant fibrosis in chronic HCV patients[40,41]. The platelet count by itself has proven to be orientative for liver fibrosis[42].

In a study made on 191 HCV infected patients compared the negative and positive predictive values for APRI and ELF™[43]. They performed liver biopsy and used cut-offs for the APRI score of ≤0.42 and ≥1.2, for the separation of mild fibrosis and significant fibrosis. APRI was an excellent predictor in chronic infected patient population.

A review by Stauber and Lackner comparing different non-invasive tests for liver fibrosis in chronic

hepatitis C patients, found APRI with an area under ROC curve of 0.88, was a better predictor for fibrosis when compared to other tests[44].

We classified liver fibrosis by Fibroscan in mild to moderate (F0-F2) and advanced/severe fibrosis to liver cirrhosis (F3-F4). A cut-off value of 0.5 for APRI score predicted successfully liver fibrosis with area under ROC curve pretty good (0.75). APRI score could differentiate the patients with high risk to have cirrhosis and exclude the ones with no or mild fibrosis.

CONCLUSIONS

In limited resources countries, due to high costs of DAAs, we are still in need to find more criteria to define which are the patients that can benefit from therapy with Peg-IFN and RBV.

Genetic determinism of HCV infection impact on the evolution of liver disease and the quality of patient's life and influence on the therapeutic decision based on the real and actual possibilities.

In countries with limited financial resources, curing/eradicating chronic hepatitis C is still a distant goal. As a new era of DAAs has come, it is important to make them available to the patients that are in need.

We could manage treating all our HCV infected population by combining various predictive factors like failure of previous IFN therapy, interleukin's genetic variation, liver fibrosis, HCV RNA load before treatment, patient age and comorbidities.

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