Research Article

Predictors of Sustained Virological Response in Chronic Hepatitis C Patients Treated with Current Direct Acting Antiviral Drugs.

Elwy MK Soliman*, Hisham AA Morsy,
Ashraf MM Othman and Ahmed M Mady***
* Department of Internal Medicine, , Faculty of Medicine, Minia University, Minya, Egypt
**Department of Clinical Pathology, Faculty of Medicine, Minia University, Minya, Egypt

Abstract
Background and aims: Treatment of hepatitis C virus (HCV) changed dramatically with the introduction of oral direct-acting antiviral drugs due to their high antiviral potency and safety profile. Sofosbuvir plus daclatasvir combination therapy was extensively investigated in HCV genotypes 1, 2, and 3, while published data regarding its real-life application in the treatment of genotype 4 is lacking. Therefore, we conducted this study to assess the outcomes and predictors of treatment response with sofosbuvir plus daclatasvir with or without ribavirin in Egyptian patients with genotype 4 hepatitis C virus infection. **Patients and methods:** This prospective study included 200 Egyptian patients with chronic genotype 4 HCV, treated with sofosbuvir plus daclatasvir with or without ribavirin for 12 weeks. Evaluation of number of non-responders, their demographics, and evaluation of C-X-C motif chemokine- 10(CXCL-10) level, interleukin 12 (IL 12) and natural killer (NK) cell phenotype pretreatment and12 weeks of treatment. **Results:** A total of 92.5% of all patients achieved SVR12. SVR12 rates of 96.29% and 84.61% were reported in non-cirrhotic and cirrhotic patients, respectively. Older age, cirrhosis, low platelet count, high level of CXC-L 10 , lower NK cell frequency and lower frequency of the NK subset $CD56^{-}CD16^{+}$ were the predictors of treatment non-response. **Conclusion:** Based on this prospective study, sofosbuvir plus daclatasvir with or without ribavirin for 12 weeks appears to have favorable outcomes in the treatment of genotype 4 HCV-infected Egyptian patients. Older age, cirrhosis, and low platelet count, high level of CXC-L 10 , lower NK cell frequency and lower frequency of the NK subset $CD56^{-}CD16^{+}$ are independent risk factors of treatment non-response.
Keywords: hepatitis C virus, genotype 4, sofosbuvir plus daclatasvir, sustained virologic response

Introduction
Hepatitis C virus (HCV) infection is a global public health problem affecting~184,000,000 people worldwide.[1] In Egypt, the prevalence of HCV infection among general population was estimated to be 15%; >90% of the infection was reported to be genotype 4[2,3]. With the advent of directly acting antiviral (DAA) therapy for hepatitis C virus (HCV), there has been a great increase in the number of patients who can expect to achieve sustained virological response (SVR). In contrast to the historical treatment of pegylated interferon (IFN) and ribavirin, DAAs deliver SVR rates in the order of 90%–95% and higher[4,5]. Little is known about predictors of failure to achieve SVR with DAAs. Although numerous parameters predicted poor response to pegylated IFN treatment (eg, age, ethnicity, human immunodeficiency virus [HIV] co-infection, insulin resistance, and interleukin [IL]-28b genotype), none of them have been shown to be associated with virological relapse after DAA based therapy[4,6,7]. There is evidence that the presence of cirrhosis still has an impact on the likelihood of SVR[8]. In the recent decade, liver stiffness (LS) determination by means of transient elastometry has become a widely accepted method for the evaluation of liver fibrosis in HCV-infected patients[9,10].

Clinical trials and studies include patients with an LS above a specific threshold, commonly >12.5–14.6 kPa, to define a sub-population bearing cirrhosis. LS also has a predictive capacity for the presence of portal hypertension[11,12] and different levels of LS are

strongly associated with the clinical outcome of cirrhosis.[13] However, the median levels of LS differ considerably between clinical trials and studies aimed at evaluating the efficacy and safety of therapy against HCV infection in patients with cirrhosis. In addition, response according to the level of LS have scarcely been analysed in cirrhotic subjects receiving DAA-based combinations, in spite of the fact that the degree of LS was independently associated with the likelihood to achieve SVR to dual therapy with Peg-IFN/RBV within this subset[14]. One of the hallmarks of HCV persistence is the failure of both innate and adaptive antiviral immune responses to clear HCV. This results in continual immune activity in the presence of ongoing viremia. Hepatitis C virus ribonucleic acid (RNA) is detected in host cells by Toll-like receptor 3 (TLR-3) or cytosolic RIG-1 helicase-mediated pathways, leading to transcriptional activation of type 1 IFN. Type 1 IFN binds to the cell surface receptor and activates the Jak-STAT pathway, which induces transcription of IFN-stimulated genes (ISGs), which have antiviral activity[15]. During chronic HCV infection, viral replication is sustained despite persistently high ISG expression. Increased type 1 IFNs also activate natural killer (NK) cells, which, in the context of HCV, display a polarized phenotype with increased cyto-toxicity, proapoptotic TRAIL production, and decreased cytokine production[16].

The CD56−CD16+ subset of NK cells is enriched in patients with HCV infection and is a dysfunctional subset with impaired cytotoxicity and cytokine production and a loss of poly-functionality likened to exhaustion seen in T cells[17].Persistent antigenic stimulation results in T-cell exhaustion with a sequential loss of antiviral function. The innate immune response contributes to the inadequacy of the adaptive response, because abrogation of IFN signaling in animal models reduces T-cell exhaustion[18]. Features of the specific immune response seen in chronic HCV have been associated with a poor response to pegylated IFN[19-21]. Whether they are also associated with the response to DAAs has not yet been ascertained. CXCL-10 is a chemokine known to be released from HCV infected livers as a result of increased ISG expression. Serum CXCL-10 is highly corre-lated with intrahepatic CXCL- 10 mRNA expression[19] and is often used as a surrogate marker of hepatic ISG expression[9]. Thus, increased serum CXCL-10 and elevated pre-treatment ISG expression are both predictors of nonresponse to pegylated IFN-based treatment[22,23].

Patients and methods

This prospective study was conducted in outpatient clinics of Minia university liver center, Egypt. A total of 200 patients with chronic HCV infection were recruited from the outpatient clinics during the period from January 2018 to May 2019.

Approval of the Institutional Ethics Committee of Faculty of Medicine, Minia University, Egypt, was obtained prior to the start of the study. A written informed consent was signed by each patient prior to enrollment. All authors had access to the study data and reviewed and approved the final manuscript.

Patients with the following criteria were included in this study: age >18 years, positive HCV antibodies con- firmed with a positive polymerase chain reaction (PCR) for HCV-RNA, treatment-naive, and Child–Pugh score >7. Pregnant females, patients with renal impairment (serum creatinine >2.5 mg/dL and estimated glomerular filtration rate <30 mL/min/ 1.73 m^2), patients with HCC (unless there was no evidence of activity by dynamic imaging 12 weeks after successful curative treatment) and patients with hepatitis B virus or human immunodeficiency virus co-infection were excluded from the study.

Pre-treatment measures:

All patients were subjected to the following: thorough history taking, clinical examination, complete blood count (CBC), liver function tests (aspartate transaminase, alanine transaminase, serum bilirubin, serum albumin, and interna-tional normalized ratio), serum creatinine, HCV antibody, HBs-Ag, α-fetoprotein, and abdominal ultrasound. Liver cirrhosis was confirmed by fibroscan and/or Fibrosis-4 (FIB-4) Index for Liver Fibrosis >3.25 assessment. Estimation of HCV RNA level was done by Cobas Ampli Prep/Cobas TaqMan HCV-RNA assay (Roche Diagnostics; Pleasanton, CA, USA) with a threshold of detection 15 IU/mL, plasma

cytokine levels: IL12, CXCL-10, and blood immunophenotyping of total PBMC to quantify NK cell frequency and subtypes, NK and its subtypes were defined by surface expression of of CD 56 and CD16

Treatment regimens:
Non-cirrhotic naïve patients were treated with sofosbuvir (Soflanork, Mash Company, Cairo, Egypt; 400mg, orally, once daily) plus daclatasvir (Daklanork, Mash company, Egypt; 60 mg, orally, once daily) for 12 weeks. Weight-based ribavirin (Ribovinol, Mash Company; 1200 or 1000 mg/day if <75 or >75 kg body weight, respectively) was added to this regimen when treating cirrhotic patients.

During treatment:
Adherence data was gathered by questionnaire, patient self-report, and pill count at each clinic visit.
Follow-up was done by clinical assessment of the patients and reviewing the results of laboratory tests (CBC, liver function tests, and renal function tests) at weeks 4, 8, and 12 of the treatment. plasma cytokine levels: IL12, CXCL-10, and blood immunophenotyping of total PBMC to quantify NK cell frequency and subtypes were done at 12 weeks of treatment.

Post treatment:
Quantitative real-time PCR for HCV RNA was done at 12 weeks post-treatment to confirm SVR. The effectiveness of sofosbuvir plus daclatasvir with or with- out ribavirin was measured by the number of patients with successful elimination of the virus, illustrated by sustained virologic response at 12 weeks after the end of treatment (SVR12). SVR12 was defined as undetectable HCV-RNA (<15 IU/mL) at 12 weeks after the end of treatment. While failure to achieve SVR –either relapse or breakthrough – was defined as nonresponse

Statistical analysis
Continuous variables are expressed as medians (interquartile range). Continuous variables were compared using Mann- Witney U test, and discreet variables were compared using Fisher's exact test. Univariate logistic analysis was carried out with treatment outcome as the dependent variable; variables that were significant in univariate were included in multivariate logistic analysis. P values of <.05 were deemed statistically significant. All statistical analysis was carried out using SPSS (IBM SPSS Statistics V23.0).

Results
Two hundred patients with chronic HCV infection were enrolled in this study. The baseline demographic and laboratory data are shown in Table 1.

Table 1: Baseline demographic and laboratory data of the studied patients

Variables		Number (200)	%
Age (years)	**Mean±SD**	49.73±10.97	
Sex	**Male**	118	59
	Female	82	41
Liver status	**Non-cirrhotic**	135	67.5
	Cirrhotic (Child A)	65	32.5
History of diabetes mellitus	**Non-diabetic**	152	76
	Diabetic	48	24
Hb (g/dL)	**Mean±SD**	13.26±1.71	
WBC (×103)/mm3	**Mean±SD**	6.44±2.15	
Platelet (×103)/mm3	**Mean±SD**	207.27±92.88	
ALT (IU/L)	**Mean±SD**	57.39±42.93	
AST (IU/L)	**Mean±SD**	57.54±32.93	
Serum bilirubin (mg/dL)	**Mean±SD**	1.36±0.40	
Serum albumin (mg/dL)	**Mean±SD**	4.12±0.54	
INR	**Mean±SD**	1.51±0.22	

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Hb, hemoglobin; INR, international normalized ratio; WBC, white blood cells.

The outcome of sofosbuvir plus daclatasvir with or without ribavirin at 12 weeks was detected by SVR12 as demonstrated in Table 2.

Table 2: Outcome of treatment in the studied patients (sustained virologic response at 12 weeks)

Variables	Number of the studied patients	Sustained virologic response at 12 weeks	
		N	%
Overall patients	200	185	92.5
Non-cirrhotic patients	135	130	96.29
Cirrhotic patients	65	55	84.61

Analyses of factors that could have affected the response to treatment revealed that older age, liver cirrhosis, and low platelet count were the factors that were significantly associated with non-response to treatment as shown in Table 3.

Also high level of pretreatment CXCL-10 could predict non-response, as non-responders had higher median pretreatment CXCL-10 levels; 320 pg/mL (179- 461) compared with 109 pg/mL (88-170) in responders ($P < .001$), and the baseline NK phenotype differed between

responders and non-responders. Responders demonstrated a significantly higher NK cell frequency: 7.01% (4.3- 7.9) vs 4.3% (2.9- 5.3) in non-responders (p = .018). Responders also had significantly higher frequencies of the NK subset $CD56^{-}$ $CD16^{+}$ (P = .004). The dynamics of CXCL-10, NK cell frequencies and phenotype during directly acting antiviral treatment differed between responders and non-responders as the following:

CXCL-10 was significantly higher in non-responders than in responders both at baseline and at the end of treatment: 215 pg/ml (115-378) compared with 93 pg/ml (44-166) in responders. Natural killer cell frequency was higher at baseline in responders and baseline frequencies of the NK cell subset $CD\ 56^{-}CD16^{+}$ were significantly higher in responders 5.1% (range, 2.5 -8.5) than non-responders 2.1% (range, 1.2-3.1) (p = .004). The frequency of the $CD56^{-}CD16^{+}$ population decreased over the course of treatment in responders but increased in non-responders so that by end of treatment, numbers were similar: 4.0% (2.5- 8.5) in responders and 3.8% (2.2- 5.1) in non-responders. There was no difference in the level of IL12 at baseline or at the end of treatment between responders and non-responders.

Table 3: Predictors of non-response to therapy in the studied patients

Variables		Responders (N=184)		Non-responders (N=16)		*P*-value
		N	%	N	%	
Age (years)	**Mean±SD**	49.38±11.14		53.79±7.72		0.01*
Sex	**Male**	109	59.23	9	56.25	0.63
	Female	75	40.76	7	43.75	
Liver status	**Non-cirrhotic**	130	70.65	5	31.25	0.0002*
	Cirrhotic (Child A)	54	29.34	11	62.75	
History of diabetes mellitus	**Non-diabetic**	142	77.1	10	62.5	0.17
	Diabetic	42	22.8	6	37.5	
Hb (g/dL)	**Mean±SD**	13.29±1.72		12.82±1.63		0.20
WBC (×103)/mm3	**Mean±SD**	6.49±2.18		5.84±1.74		0.13
Platelet (×103)/mm3	**Mean±SD**	210.41±94.66		171.15±59.29		0.02[a]
ALT (IU/L)	**Mean±SD**	58.44±44.19		45.22±21.94		0.26
AST (IU/L)	**Mean±SD**	57.54±33.18		57.44±30.58		0.89
HCV viral load	**Mean±SD**	1019150.9±3559000.2		1190846.5±1846267.7		0.15

[a]**Notes:** Significant.
*Statistically significant at $P \leq 0.05$.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HCV, hepatitis C virus; Hb, hemoglobin; WBC, white blood cells.

Discussion

In our study, non-cirrhotic naïve patients were treated with sofosbuvir plus daclatasvir for 12 weeks. Ribavirin was added to this regimen when treating cirrhotic patients. One hundred and eighty five (92.5%) patients achieve successful eradication of HVC. SVR 12 was 96.29% and 84.61 in non-cirrhotic and cirrhotic patients respectively. Our results were in accordance with Fontaine et al., who concluded that combination of sofosbuvir and daclatasvir was associated with a high rate of SVR in treatment of genotype 4 HCV[24]. Forty-seven patients with genotype 4 HCV were enrolled in their study and received a combination of sofosbuvir and daclatasvir with or without ribavirin for 12 or 24 weeks, respectively. The overall SVR was 86%–100%, according to patients' baseline characteristics and therapeutic regimen. They also concluded that there was a beneficial effect in treatment-experienced and cirrhotic patients when either ribavirin was added or treatment duration was extended from 12 to 24 weeks, and the combination of sofosbuvir plus daclatasvir was generally well tolerated with mild adverse events. In a recent Egyptian study including <18,000 patients with HCV infection, about 95% achieved SVR12. It was concluded that this regimen is safe and effective for the treatment of Egyptian patients with chronic hepatitis C genotype 4[25].

Our results were in agreement with the study performed by Pol et al., who documented that combination of sofosbuvir and daclatasvir had high antiviral potency, with >90% SVR rate in patients with chronic HCV infection[26]. With regard to the predictive factors associated with non-response to therapy, various host and viral variables (e.g., gender, age, race, body mass index, insulin resistance, steatosis, advanced fibrosis stage, HCV genotype, and viral load) had been well identified and were associated with nonresponse to interferon based therapies[27-29]. There are few studies that have described the characteristics of individuals who fail to respond to DAAs, and the majority of these have focused on HCV viral resistance[30-31].

Our results revealed that older age, cirrhosis, and low platelet count and a number of immunological parameters, CXCL-10 serum levels and NK immunophenotype were the predictors of non-response associated with sofosbuvir and daclatasvir therapy for genotype 4 HCV among Egyptian patients. This might be attributed to the fact that most of the patients with older age and/or low platelet count in our study were associated with liver cirrhosis at presentation likely caused by a longer duration of HCV infection.

With regard to age, few studies showed the relation of older age to SVR rates using all oral DAA regimens because elderly patients were often excluded from clinical trials. However, little differences in SVR rates were observed between elderly patients and younger ones[32-33]. With regard to liver status, Ferenci et al., reported that the severity of hepatic dysfunction appeared to affect the response rate to DAA, with higher SVR in patients with chronic hepatitis or Child A liver cirrhosis than in those with Child B or C liver cirrhosis[34]. Our findings that elevated CXCL-10 at baseline is associated with non-response was in agreement with Childs et al.,[35], but upregulation of CXCL-10 on treatment is associated with response is directly analogous to the well reported mechanism of ISG expression as a predictor of response to pegylated IFN[21]. That IFN signaling still plays a role even in IFN-free treatment fits with hepatic gene expression data from Meissner et al.,[36], who showed that hepatic IFN-α expression increased during successful IFN free treatment for HCV. At the end of 12 weeks of treatment, a cross-sectional comparison between responders and non-responders showed that hepatic ISG expression was higher in responders[36]. These authors suggest an ongoing role for IFN signaling even during DAA therapy for HCV. We found a significant elevation in CXCL-10 level at the end of treatment in non-responders this is in agreement with Child et al.,[35]. This may represent an ongoing innate immune response to low-level residual HCV viremia before overt virological relapse. As regard NK cell phenotype in agreement with Childs et al., we found that responders had a higher frequency of the CD56−CD16+ NK cells subset [35]. This was an unexpected finding. The CD56−CD16+ subset of NK cells is enriched in patients with HIV and HCV infection and is a dysfunctional subset with impaired cytotoxicity and cytokine production and a loss of polyfunctionality likened to exhaustion seen in T cells[19].

The limitation of our study that Child B and C patients were not included, who might be less likely to have a response. When investigating the immunological predictors, we confined to investigating the peripheral immune response rather than the hepatic response as peripheral NK frequency and phenotype may not be a mirror for intrahepatic NK populations. Also other variable cytokines and immune cell should be further investigated.

Conclusions

Based on this prospective study, combined sofosbuvir plus daclatasvir with or without ribavirin for 12 weeks appears to have favorable outcomes with high rates of SVR and safety profile in the treatment of Egyptian patients with genotype 4 HCV infection. Older age, cirrhosis, and low platelet count, high serum level of CXCL-10 and low frequency of NK subset CD56- CD16+ are independent risk factors of treatment non-response. Sofosbuvir plus daclatasvir regimen should be considered in the treatment of genotype 4 HCV-infected patients. Large-scale studies of sofosbuvir plus daclatasvir for the treatment of chronic HCV, particularly in the so-called "difficult-to-treat" patients, are recommended.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epi- demiology of hepatitis C virus infection: new esti- mates of age specific antibody to HCV seroprevalence. Hepatology. 2013; 57: 1333–1342.
2. Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: results from a nationwide survey. J Viral Hepat. 2012;19:560–567.
3. Abd-Elsalam S, Sharaf-Eldin M, Soliman S, Elfert A, Badawi R, Ahmad YK. Efficacy and safety of sofosbuvir plus ribavirin for treatment of cirrhotic patients with genotype 4 hepatitis C virus in real-life clinical practice. Arch Virol. Epub 2017; 163:51–56.
4. Afdhal N, Zeuzem S, Kwo P, et al.,. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014; 370:1889–98.
5. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al., Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014; 370:211–21.
6. Berry L, Irving W. Predictors of hepatitis C treatment response: what's new? Expert Rev Anti Infect Ther 2014; 12:183–91.
7. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. J Hepatol 2017; 66:153–9
8. Afdhal N, Reddy KR, Nelson DR et al., (2014) Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med 370:1483–1493
9. Sandrin L, Fourquet B, Hasquenoph JM et al., (2003) Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 29: 1705–1713
10. Vergara S, Macías J, Rivero A et al., (2007) The use of transient elastometry for assessing liver fibrosis in patients with HIV and hepatitis C virus coinfection. Clin Infect Dis 45:969–974
11. Pineda JA, Recio E, Camacho A et al., (2009) Liver stiffness as a predictor of esophageal varices requiring therapy in HIV/hepatitis C virus-coinfected patients with cirrhosis. J Acquir Immune Defic Syndr 51:445–459
12. Mandorfer M, Kozbial K, Schwabl P et al., (2016) Sustained virological response to interferon-free therapies ameliorates HCVinduced portal hypertension. J Hepatol 65:692–699
13. Merchante N, Rivero-Juárez A, Téllez F et al., (2012) Liver stiffness predicts clinical outcome in human immunodeficiency virus/hepatitis C virus-coinfected patients with compensated liver cirrhosis. Hepatology 56:228–23
14. Mira JA, García-Rey S, Rivero A et al., (2012) Response to pegylated interferon plus ribavirin among HIV/hepatitis C virus coinfected patients with compensated liver cirrhosis. Clin Infect Dis 55:1719–1726
15. Curry MP, O'Leary JG, Bzowej N et al., (2015) Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis.

N Engl J Med 373:2618–2628
16. Poordad F, Schiff ER, Vierling JM et al., (2016) Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. Hepatology 63: 1493–1505
17. Meylan E, Curran J, Hofmann K, et al., Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus.Nature 2005;437:1167–72
18. Golden-Mason L, Rosen HR. Natural killer cells: multifaceted players with key roles in hepatitis C immunity. Immunol Rev 2013; 255:68–81.
19. Bjorkstrom NK, Ljunggren HG, Sandberg JK. CD56 negative NK cells: origin, function, and role in chronic viral disease. Trends Immunol 2010; 31:401–6.
20. Teijaro JR, Ng C, Lee AM, et al.,. Persistent LCMV infection is controlled by blockade of type I interferon signaling. Science 2013; 340:207–11.
21. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, et al., Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008; 105:7034–9.
22. Oliviero B, Mele D, Degasperi E, et al., Natural killer cell dynamic profile is associated with treatment outcome in patients with chronic HCV infection. J Hepatol 2013; 59:38–44.
23. Feld JJ, Nanda S, Huang Y, et al., Hepatic gene expression during treatment with peginterferon and ribavirin: identifying molecular pathways for treatment response. Hepatology 2007; 46:1548–63.
24. Fontaine H, Hezode C, Zoulim F, et al., Efficacy of the oral sofosbuvir based combinations in HCV genotype 4-monoinfected patients from the French observational cohort ANRS CO22 Hepather. Abstract LP28 presented at: 50th Annual Meeting of European Association for the Study of the Liver; April 22–26, 2015; Vienna, Austria.
25. Omar H, El Akel W, Elbaz T, et al.,. Generic daclatasvir plus sofosbuvir, with or without ribavirin, in treatment of chronic hepatitis C: realworld results from 18 378 patients in Egypt. Aliment Pharmacol Ther. 2018;47(3):421–431.
26. Pol S, Corouge M, vallet-Pichard A. Daclatasvir–sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: from the clinical trials to real life. Hepat Med. 2016;8:21–26.
27. Afdhal NH, McHutchison JG, Zeuzem S, et al., Hepatitis C pharmacogenetics: state of the art in 2010. Hepatology. 2011; 53: 336–345.
28. Hadziyannis SJ, Sette H, Morgan TR, et al.,. Peginterferon alpha 2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346–355.
29. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut. 2006;55:1350–1359.
30. Gozlan Y, Ben-Ari Z, Moscona R, et al.,. HCV genotype-1 subtypes and resistance-associated substitutions in drug-naive and in direct-acting antiviral treatment failure patients. Antivir Ther 2017. doi: 10.3851/IMP3123.
31. Wyles D, Dvory-Sobol H, Svarovskaia ES, et al.,. Post-treatment resistance analysis of hepatitis C virus from phase II and III clinical trials of edipasvir/sofosbuvir. J Hepatol 2017; 66:703–10.
32. Reid M, Price JC, Tien PC. Hepatitis C virus infection in the older patient. Infect Dis Clin N Am. 2017;31:827–838.
33. Saab S, Park SH, Mizokami M, et al.,. Safety and efficacy of ledipasvir/ sofosbuvir for the treatment of genotype 1 hepatitis C in subjects aged 65 year or older. Hepatology. 2016;63(4):1112–1119.
34. Ferenci P, Kozbial K, Mandorfer M, Hofer H. HCV targeting of patients with cirrhosis. J Hepatol. 2015;63:1015–1022.
35. Childs, K., Merritt, E., Considine, A., Sanchez-Fueyo, A., Agarwal, K., Martinez-Llordella, M., & Carey, I. (2017). Immunological predictors of nonresponse to directly acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. In Open forum infectious diseases (Vol. 4, No. 2, p. ofx067). US: Oxford University Press.
36. Meissner EG, Wu D, Osinusi A, et al.,. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. J Clin Invest 2014; 124:3352–63.