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Impact of DAA Therapy in HCV on Thrombocytopenia and Fibrosis : A prospective study in Tertiary Care Centre

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Abstract: *Hepatitis C virus (HCV), a primary aetiology of chronic liver disease that can lead to cirrhosis and Hepatocellular cancer (HCC), infects an estimated 70 million individuals globally. Despite the fact that modern antiviral treatment, which includes direct acting antivirals (DAA), may yield a very high sustained virological response rate. Essential role of Platelets in liver infections, which help to reduce liver fibrosis by inactivating hepatic stellate cells, which reduces collagen formation. This study evaluated the impact of Direct Acting Antivirals (DAA) on Thrombocytopenia and Fibrosis. Evaluate the DAA treatment response in progression of fibrosis, respective Genotypes along with Sustainable Virological Response (SVR) in HCV patients.results were Obtained from treated patients with DAA, SVR was not associated with considerable regression of fibrosis, also no significant improvement in platelet count in F1-F3 fibrosis even though patients achieved SVR. Genotype 3 patients with compensated cirrhosis did not achieve SVR with DAA therapy.*

Key Words: *DAA; Direct acting antivirals, SVR; Sustainable Virological Response and HCV; hepatitis C virus, thrombocytopenia.*

1. INTRODUCTION :

Hepatitis C virus (HCV), a primary aetiology of chronic liver disease that can lead to cirrhosis and hepatocellular cancer(HCC), infects an estimated 70 million individuals globally (1). Which is a tiny encapsulated RNA virus from the Flaviviridae family and the genus Hepacivirus. (2). HCV genomic RNA is a single-stranded, positive-polarity RNA that is packed by core protein and encased by a lipid bilayer including two viral glycoproteins (E1 and E2) to create the virion. (3). The most frequent HCV is genotype 1, which accounts for 83.4 million cases (46.2 percent of all HCV infections), with almost a third of those in East Asia. Genotype 3 is the world's second most frequent genotype (54.3 million, or 30.1 percent); genotypes 2, 4, and 6 account for 22.8 percent of all cases; and genotype 5 makes up the remaining 1%. (10).direct-acting antiviral (DAA) is for the treatment of HCV infection, an early introduction of oral DAA that target three separate HCV non-structural (NS) proteins has led in considerable advances in the safety and efficacy of therapies that cure HCV infection (sustained virologic response (SVR rates in excess of 95%.)). also reduces the risk of HCC develop but not completely, even after achieve of SVR (5). Current findings argue strongly in favour of early treatment before the development of cirrhosis and in implementing or continuing HCC surveillance among patients with cirrhosis even after achieving SVR(6). Platelets play a key role by carrying proteins which aid in hemostasis as well as a variety of growth factors that aid in organ development, tissue regeneration, and repair. Thrombocytopenia can be induced by reduced thrombopoietin production and faster platelet destruction caused by hypersplenism, which is seen in 64 percent to 84 percent of individuals with chronic liver disease (CLD) and cirrhosis(7). In studies, platelets have been shown to improve liver fibrosis and accelerate liver regeneration (8). Thrombocytopenia is associated with an increased hepatic venous pressure gradient (HVPG) (9).

Patients with HCV infection are commonly asymptomatic and incidentally diagnosed while undergoing blood investigations for some other procedure. Symptomatic patients presented with nausea, abdominal pain, flu like symptom and mild transaminitis. In most individuals, HCV RNA is usually detectable within two weeks and anti HCV antibodies within 3 to 12 weeks of exposure of HCV(11). Serum alanine aminotransferase (ALT) levels usually rise within 8–10 weeks, with a peak ALT of 10–20 times the upper limit of normal. Serum HCV RNA levels may fluctuate widely during the acute phase and even become negative transiently, only to reappear again. This finding is only seen in the acute phase and may be a clinical clue to the diagnosis of acute HCV infection. Spontaneous resolution occurs in 15–25% of subjects and may be up to 45% in persons who present with jaundice, children, and

young women. Higher rates of spontaneous clearance were also observed in persons with certain polymorphisms (the rs12979860-C,[5] rs8099917-T[6] and the ss469415590 TT) near to the IL-28B gene (interferon lambda)(12). HLA class II alleles may play a role in spontaneous clearance. Less genetic diversity of the viral E1 and E2 envelope genes were observed in subjects with spontaneous recovery compared to those who progressed to chronic infection(13). The previous therapeutic history of HCV by the Interferon-based regimens, and later with addition of ribavirin (RBV), were the standard of care for many years for patients with chronic hepatitis C treatment (CHC). However, treatment end results diverse between genotypes, with particularly poor cure rates of 40% being reported in GT1 and GT4 cases. (14) Many directly acting antivirals (DAAs) have been approved for use as part of CHCV combination therapies since 2011, and patient outcomes have significantly improved(15). In this study followed up of Patients who were treated with direct acting antivirals (DAA) for HCV, assess treatment response in progression of fibrosis in HCV patients with Sustainable Virological Response (SVR) in respective Genotypes 1,2,3,4, 5 & 6.

2. METHODS:

2.1 Study Design: In this Prospective longitudinal studyHCV patients who were treated with DAA for a period between January 2016 and December 2018 at Institute of hepato-biliary sciences, Madras Medical College, Chennai, Tamil Nadu, India were included.

Treatment duration was determined by the cirrhosis and non-cirrhosis condition of the patients. No patients received ribavirin along with DAA. All patients who were treated with DAA were Followed up for a period of 36 months. The study ended on the 31st December 2018.

2.2 Study population: Study population included 807 patients attended above outpatient clinic with chronic HCV infection. Sample size was determined using online tool https://openepi.com › Sample Size. Patients who had HBV, HSV, CMV, Wilsons Disease and HIV Co-infections were excluded from this study. Patients with fibrosis stages (F1-F3) were taken in to the study.

2.3 Data Collection: All patients attended the outpatient clinic at Institute of Hepato-Biliary Sciences, Madras Medical College, Chennai were screened for anti-HCV, HBsAg through ELISA method. Among 807 Patients who tested anti-HCV positive, 302 patients startedon DAA were followed up for a period of 2 years whether they achieved SVR or not. Patients were monitored with complete blood count, liver function tests, USG Abdomen and liver stiffness. Informed consent was got from the patients before starting the treatment.All aspects of the treatment and adverse events were explained to the patients.

2.4 Inclusion Criteria: Patients with fibrosis (F1,F2 and F3)due to Chronic HCV Infection diagnosed by Fibroscan alone were included in the study.

2.5 Exclusion Criteria: Patients with Chronic HBV and HCV Co-infection, Chronic HCV and HIV Co-infection, HSV, CMV, Non-cirrhotic HCV Patients, Compensated Cirrhosis due to HCV, DCLD due to HCV and ALDwere excluded from the study.

2.6 Sample collection and analysis: 3 ml of blood sample was collected from the patients who attended outpatient clinic. Screening of anti HCV and HBsAg was performed through by ELISA Method (Transasia Bio Medicals Ltd test kit). Sensitivity and Specificity of HCV ELISA were 97.6% and 92.6%. Circulating blood cells, include red blood cells, white blood cells are counted and sized electronically by Sysmex XN 1000 fully automated 5 part hematology analyser(16) and LFT was performed in Erba-EM360 fully automated analyser(17). HCV RNA quantitative analysis is performed by RT PCR method by SRL Diagnostics, Chennai.

2.7 End Points: The Primary Efficacy end point was Sustainable Virological Response (SVR), defined as an undetectable HCV RNA viral Load 12 weeks after treatment completion(18). Patients with detectable HCV RNA at the SVR time point were considered to have viral relapse. Secondary endpoints included efficacy of DAA in the improvement of the fibrosis status(19).

2.8 Data Analysis: With 302 patients, the 95% CI for SVR was expected no more than 2.4% in both directions on the basis of a hypothesized 90% SVR. The primary analysis of efficacy was the proportion of overall patients who achieved SVR with a 2 sided 95% CI.

All the data collected were entered in MS Excel Sheet. Analysis done by SPSS 20.0. Quantitative data expressed in mean and standard deviation. Qualitative data was analysed through univariate and multivariate logistic regression analysis with 95 % CI and statistically significant was assessed by $P<0.05$. Independent variables were analysed through student t test.

2.9 Treatment: Study patients were treated with Sofosbuvir –NS5B polymerase inhibitor(8), Daclatasvir – NS5A polymerase inhibitor(20), Ledipasvir- NS5A polymerase inhibitor,Velpatasvir- NS5A polymerase inhibitor which was provided by the tertiary care centre(21).Genotype 1 and 4 treated with Sofosbuvir 400mg and Ledipasvir

90mg.Genotype2 and 3 with Sofosbuvir 400mg and Daclatasvir 60mg. Sofosbuvir 400mg and Velpatasvir 100mg as pangenotype antiviral treatment irrespective of genotype and monitoring the haematological, biochemical parameters. The Primary Efficacy end point was SVR, defined as an undetectable HCV RNA viral load 12 weeks after treatment completion. Patients with detectable HCV RNA at the SVR time point were considered to have viral relapse. Secondary endpoints included efficacy by subgroups: Genotypes, fibrosis status and cirrhosis. Cirrhosis was determined by USG abdomen andFibroscan.

2.10 Intervention and Follow up: All the 302 Patients who were treated with direct acting antivirals (DAA) for HCV followed up for 36 months.

2.11 Study Oversight: This study was intramurally funded by Institute of Hepato-biliary sciences, Madras Medical College, Chennai; and conducted in compliance with the provisions and declarations of AASLD practise guidelines and local regulatory requirements.

3. RESULTS:

Among total 807 patients 463 (57.3%) patients were male and 344 (42.6%) were female. Average age of male was 42.41 (±16.69) and female was 44.22(±16.01). 261 (32.3%) patients had history of surgery and 232 (28.7%) patients had a historyof blood transfusion. 111 (13.75%) patients had hemodialysis for Chronic Kidney Disease. 111 (13.75%) patients had Diabetes Mellitus. 112 (13.8%) patients had systemic hypertension (Table 1). The percentage of fibrosis progression 26.1% is increased than fibrosis regression (13.9%), 44.7%patient remained in F4 fibrosis stage and 15.2 % patients remained in F1 to F3 fibrosis. Table 1.

Table1. The Percentage of fibrosis progression

Fibroscan Stages	**No. of patients (%)**
F1-F2	48 (39.66)
F1-F3	5 (4.13)
F1-F4	5 (4.13)
F3-F4	10 (8.2)
F2-F3	10 (8.2)
F2-F4	1 (0.82)
F4-F2	9 (7.4)
F4-F3	17 (14.04)
F2-F1	2 (1.52)
F3-F1	4 (3.3)
F4-F1	0
F3-F2	10 (8.2)
Total	121

Table 1: The percentage of fibrosis progression 79 (26.1) is increased than fibrosis regression 42 (13.9) 135 (44.7) patients remained in F4 fibrosis without any regression and 46 (15.2) remains stable in their respective stages (F1-F3) fibrosis.

3.1 Treatment Results of DAA: Among 807 serological positive patients 302 patients were enrolled for the treatment. Among them one hundred and sixty one (161) patients with genotype 1, five (5) patients with genotype 2, one hundred and seventeen (117) patients with genotype 3, eighteen patients (18) with genotype 4, and 1 patient with genotype 6.All of them were followed up to End Treatment Response(ETR) and SVR. Genotype 1,2,4 and 6 achieved 100% SVR for DAA. Genotype 3 achieved only 95.9% of SVR for DAA Sofosbuvir (NS5B Polymerase inhibitor) and Daclatasvir (NS5A polymerase inhibitor). The four patients of Genotype 3 with compensated cirrhosis treated with DAA for 24 weeks achieved ETR but didn't achieve SVR 12 . Out of 302 patients 135 patients were excluded due to F4 fibrosis. Remaining 167 patients with fibrosis stages (F1-F3) were followed up to 3 years after SVR (Table 2).

Table 2: **Demographics and Clinical Characteristics**

	Patients n/N	**SVR (95% CI) %.**
Over all	259/302	85.76 (83.0-88.7)
Completed Treatment	259/263	98.47 (90.3- 99.4)
Cirrhosis	64/68	94.11 (93.5-97.8)

Non Cirrhosis	195/195	100 (92.3-100.0)
Fibrosis Stage		
0	36/36	100 (92.5- 100.0)
1	69/69	100 (91.5-100.0)
2	43/43	100 (95.5- 100.0)
3	47/48	97.91 (86.2-97.1)
4	64/67	95.52 (86.5-96.8)
Treatment Duration		
12 weeks	195/195	100 (92.5-100.0)
24 weeks	64/68	94.11 (92.6-97.8)
Genotypes		
1	143/143	100 (97.8-100.0)
2	5/5	100 (98.6-100.0)
3	94/98	95.9 (86.4-98.5)
4	16/16	100(97.3- 100.0)
6	1/1	100 (92.5-100.0)

Table 2: Only four patients were treated for 24 weeks.All these four patients weren't achieve SVR 12 belonged to genotype 3 (one patient in F3 and 3 patients in F4). Cirrhosis patients has less SVR percentage (94.11%) when compare to the non-cirrhotic patients who undergone the treatment. Assessment of cirrhosis is determined by the LFT,USG Abdomen and Fibroscan.99.37% of the patients with F1-F3 achieved SVR

Table 3: **Comparison of variables before and after Treatment with DAA**

Treatment	**Stats**	**HB**			**BILIRUBIN TOTAL**			**AST**			**ALT**			**PLT**		
		BEFORE	**AFTER**	**P VALUE**	**BEFORE**	**AFTER**	**p value**	**BEFORE**	**AFTER**	**P VALUE**	**BEFORE**	**AFTER**	**p VALUE**	**BEFORE**	**AFTER**	**p VALUE**
Male	**Average**	11.03	9.8	0.152	1.14	0.49	0.025	58.4	28.3	0.017	61.6	27.7	0.023	2.04	1.7	0.02485
	Std.dev	1.97	1.89		2.3	0.62		89.5	17.3		101.88	22.36		1.12	0.8	
Female	**Mean**	11.03	10.12	0.491	0.7	0.9	0.046	89.71	27.81	0.048	83.8	28.34	0.025	1.8	1.72	0.01658
	Std.dev	1.69	1.47		1.5	1.8		169.5	16.06		159.42	20.5		0.9	1	

Table 3: Variables observed before and after the treatment - Variables Hb, Bilirubin Total, AST, ALT and PLT: There was no change in the level of haemoglobin before and after the treatment with DAA in both male (p value 0.152) and female (p value 0.491)patients. P-values of both before and after the treatment were calculated by paired t test. The level of ASTand ALT was decreased significantly after the treatment with DAA for both male (p value 0.017) and female (p value 0.048). The level of platelet was decreased and not improved even after the treatment with DAA for both male (p value 0.024) and female (p value 0.01658).

3.2 Liver stiffness: Liver stiffness measurement was performed using Fibroscan, (Echosens, Paris France) with patients in supine position and maximal abduction of right arm and transducer probe placed in the right intercostal space in the midaxillaryline. Transducer probe produces low frequency (50 Hz) elastic shear waves which traverses the liver tissue. Concordance between the liver stiffness (kPa) and fibrosis stage according to METAVIR Score was recorded and compared with the standard values of HCV(22). There was no fibrosis regression observed among the patients undergone DAA therapy. (Table 4)SWE reference kPa values for fibroscan for HCV aregiven asF0-F1 6.3-7.6, F2- 7.7-10.0, F3 -10.1- 15.6 and F4->15.6.[25]

Table 4: **FibroscanStages**

Fibroscan	Before the treatment N= 302 (%)	After the Treatment N= 302 (%)	P value
F 1	93 (30.79)	35 (11.58)	0.3996
F2	35 (11.59)	54 (17.88)	0.2565
F3	39 (12.9)	56 (18.54)	0.28671
F4	135 (44.7)	157 (51.98)	0.000596
Fibroscan(Total)	302	302	0.003

Table 4: Fibroscan stages increased F2, F3 and F4individuals after treatment of DAA in Fibroscan grade and decreased number of F1 individuals in after treatment of all genotype.

3.3 Genotype: Antiviral Therapy and treatment duration 12/24 weeks was mentioned in each patient according to the viral genotype and subtype and severity of liver disease. Among all the genotypes, Genotype 3 had less achievement in SVR. HCV RNA quantification was assessed by real time PCR, with a limit of detection of 15 IU/mL. Patients were followed up monthly with clinical and laboratory evaluation during antiviral treatment. Virological response was assessed by the quantification of HCV RNA. Virological failures and early discontinuations of therapy due to adverse events were also registered.

Table: 5 **Correlation of Fibrosis Regression/Progression before and after the treatment with DAA**

BEFORE TREATMENT		AFTER TREATMENT			
TE GRADES	TOTAL NO.OF PATIENTS (302)	F1	F2	F3	F4
F1	93	0%	48(↑ 40%)	5(↑ 4%)	5(↑ 4%)
F2	35	2(↓2%)	0%	10(↑8%)	1(↑1%)
F3	39	4(↓4%)	10(↓ 8%)	0%	10(↑8%)
F4	135	0%	9(↓ 7%)	17(↓14%)	0%

Table. 5 Correlation of Fibrosis Regression/Progression before and after the treatment with DAA: Progression of Fibrosis observed in 58(48%) (patients from F1 stage to F2, F3 and F4 in TE Grade F1) Progression observed in 11(9%) patients and regression in 2(2%) (Patients from Stages of F2 to F3 & F4 and F2 to F1 in TE Grade F2). Regression observed in14 (12%) patients and progression observed in 10 (8%) (Patientsfrom Stages F3 to F1, F2 and F3 to F4 in TE Grade F3).Regression observed in 26(21%) patients and Progression observed in F4 remains stable even after the treatment. Progression rate (65%) is higher than the regression rate (35%).

Figure 1: Fibrosis stages before and after treatment

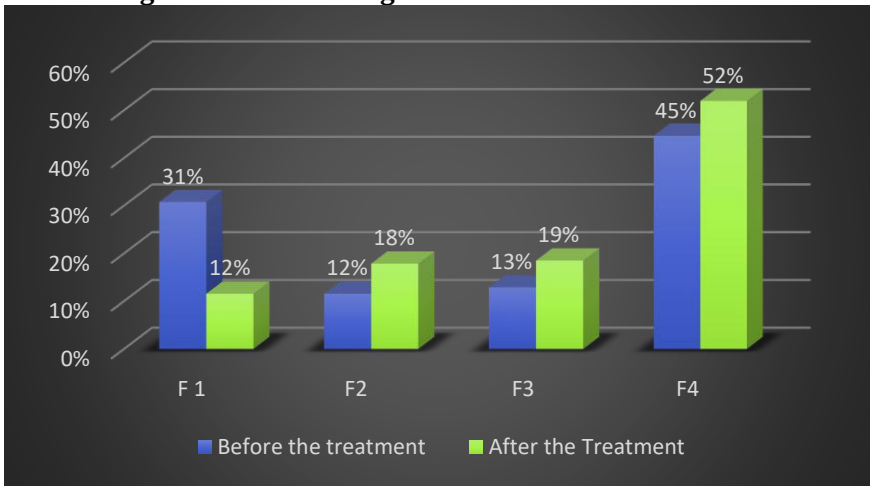

Fig 1 . Fibrosis progression is higher even after the treatment. There is significant ($p<0.05$) percentage of increase from 42% to 54% in the F4 fibrosis group.

Table 6 comparison of DAA Therapy of HCV Before and After the Treatment

	Before the treatment	*After the treatment*
Mean	14.9509934	16.060596
Variance	76.5039027	50.4197378
Observations	302	302
Pearson Correlation	0.68291646	
Hypothesized Mean Difference	0	
Df	301	
t Stat	-2.9720276	
P(T<=t) one-tail	0.00159856	
t Critical one-tail	1.64993169	
P(T<=t) two-tail	0.00319713	
t Critical two-tail	1.96787653	

4. DISCUSSION:

Direct acting antiviral have become widely used for patients with Chronic Hepatitis C virus infection(23). Consistent with other studies, the majority of patients successfully achieved viral clearance by post-treatment week 12 and 24(24). Hemoglobin, Platelet count, Liver Function Test, USG abdomen and fibroscan were taken before the treatment and same were compared at the end of the treatment, SVR 12 and during follow up. When compared to base line values, patients had no significant improvement in Platelet, fibrosis after the completion of treatment and follow up. Meanwhile there was an improvement in alanine transmainase and aspartate transaminase enzymes (table 3). In people who infected with HCV, reduced platelet generation, or thrombopoiesis, may be partially responsible for thrombocytopenia (25). One patient from F3 Fibrosis and three patients in F4 fibrosis did not achieve SVR in genotype 3 were observed. This might be due to the NS5A gene mutation. We noted excellent virological response rates in patients infected with genotype 1 HCV regardless of choice of NS5A inhibitor.

It is still controversial, whether and to what amount fibrosis and portal hypertension are reversible in patients with hepatitis C virus (HCV)-associated fibrosis and sustained virologic response (SVR) after interferon-free antiviral therapy(26). In the current study, we investigated dynamics of liver stiffness as surrogate marker of fibrosis in patients with chronic HCV infection. There is no significant regression of fibrosis after the treatment with DAA ($p < 0.05$) (Table 5).This is confirmed byliver stiffness assessment with Fibro scan before and after treatment with DAAFig 1.

167 patients with F1 to F3 fibrosis were treated with DAA and undergone SVR, fibroscan, liver profiles, hematological investigations. There is no improvement in thrombocytopenia and fibrosis. As there is no regression in fibrosis even after attaining SVR these patients should be kept under surveillance for the development of cirrhosis and HCC (Table 6) Fig 1.

5. CONCLUSION:

Among the patients treated with DAA, SVR was not associated with a considerable reduction in the progression of fibrosis. There is no improvement in the platelet count even after the treatment with DAA. 35.1 % of HCV male patients had a past history of blood transfusion and 45.7 % of female patients had history of surgery. The introduction of DAA improved the effective treatment module among the HCV patients with or without cirrhosis. This study focused on the improvement of liver fibrosis before and after the treatment of DAA on HCV patients. Patients received the DAA treatment for 12 weeks and 24 weeks. 12 weeks prescribed fornon-cirrhotic condition and 24 weeks for cirrhosis, Decompensated Cirrhosis and relapsed patients. The incidence of liver cancer would also decrease with result of repeal of chronic inflammation due to virus. Eventhough patients had achieved SVR 12 after DAA therapy as there is no significant regression ($p<0.003$) in fibrosis and thrombocytopenia ($P<0.05$)Fig 3.Hence all Patients who achieved SVR12 need HCC surveillance with LFT, alpha fetoprotein and USG abdomen lifelong. There are other factors for liver disease such as Non-Alcoholic Fatty Liver disease, alcoholic liver disease, metabolic disorders which cannot be ruled out in a patient with HCV even after achieving SVR 12.

REFERENCES:

1. Marshall, Alison D., et al. "The removal of DAA restrictions in Europe–one step closer to eliminating HCV as a major public health threat." *Journal of hepatology* 69.5 (2018): 1188-1196.

2. Chevaliez, Stéphane, and Jean-Michel Pawlotsky. "HCV genome and life cycle." *Hepatitis C viruses: genomes and molecular biology* (2006).
3. Lavie, M., Goffard, A., & Dubuisson, J. (2006). HCV glycoproteins: Assembly of a functional E1–E2 heterodimer. *Hepatitis C viruses: Genomes and molecular biology*.
4. Torres, H. A., Shigle, T. L., Hammoudi, N., Link, J. T., Samaniego, F., Kaseb, A., & Mallet, V. (2017). The oncologic burden of hepatitis C virus infection: a clinical perspective. *CA: a cancer journal for clinicians*, *67*(5), 411-431.
5. Hedenstierna, M., Nangarhari, A., Weiland, O., & Aleman, S. (2016). Diabetes and cirrhosis are risk factors for hepatocellular carcinoma after successful treatment of chronic hepatitis C. *Clinical Infectious Diseases*, *63*(6), 723-729.
6. El-Serag, H. B., Kanwal, F., Richardson, P., & Kramer, J. (2016). Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. *Hepatology*, *64*(1), 130-137.
7. Mitchell, O., Feldman, D. M., Diakow, M., & Sigal, S. H. (2016). The pathophysiology of thrombocytopenia in chronic liver disease. *Hepatic medicine: evidence and research*, *8*, 39.
8. Kurokawa, T., & Ohkohchi, N. (2017). Platelets in liver disease, cancer and regeneration. *World journal of gastroenterology*, *23*(18), 3228.
9. Groszmann, R. J., Garcia-Tsao, G., Bosch, J., Grace, N. D., Burroughs, A. K., Planas, R., ... & Makuch, R. (2005). Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *New England Journal of Medicine*, *353*(21), 2254-2261.
10. Messina, J. P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G. S., Pybus, O. G., & Barnes, E. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*, *61*(1), 77-87.
11. How Long Does It Take to Test Positive? (2021) - Viral Hepatitis and Liver Disease [Internet]. [cited 2021 Jul 2]. Available from: https://www.hepatitis.va.gov/hcv/patient/testing/time-required-to-test-positive.asp
12. Ge, D., Fellay, J., Thompson, A. J., Simon, J. S., Shianna, K. V., Urban, T. J., ... & Goldstein, D. B. (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*, *461*(7262), 399-401.
13. Farci, P., Shimoda, A., Coiana, A., Diaz, G., Peddis, G., Melpolder, J. C., ... & Alter, H. J. (2000). The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science*, *288*(5464), 339-344.
14. Burstow, N. J., Mohamed, Z., Gomaa, A. I., Sonderup, M. W., Cook, N. A., Waked, I., ... & Taylor-Robinson, S. D. (2017). Hepatitis C treatment: where are we now?. *International journal of general medicine*, *10*, 39.
15. Seifert, L. L., Perumpail, R. B., & Ahmed, A. (2015). Update on hepatitis C: direct-acting antivirals. *World journal of hepatology*, *7*(28), 2829.
16. Tefferi, A., Hanson, C. A., & Inwards, D. J. (2005, July). How to interpret and pursue an abnormal complete blood cell count in adults. In *Mayo Clinic Proceedings* (Vol. 80, No. 7, pp. 923-936). Elsevier.
17. Stockmann, M., Lock, J. F., Malinowski, M., Niehues, S. M., Seehofer, D., & Neuhaus, P. (2010). The LiMAx test: a new liver function test for predicting postoperative outcome in liver surgery. *Hpb*, *12*(2), 139-146.
18. Pockros, P. J. (2018). Management of patients who have achieved sustained virologic response for hepatitis C virus infection. *Gastroenterology & hepatology*, *14*(5), 305.
19. Jacobson, I. M., Lim, J. K., & Fried, M. W. (2017). American gastroenterological association Institute clinical practice Update—Expert review: care of patients who have achieved a sustained virologic response after antiviral therapy for chronic hepatitis C infection. *Gastroenterology*, *152*(6), 1578-1587.
20. Hézode, C., Hirschfield, G. M., Ghesquiere, W., Sievert, W., Rodriguez-Torres, M., Shafran, S. D., ... & Schnittman, S. (2015). Daclatasvir plus peginterferon alfa and ribavirin for treatment-naive chronic hepatitis C genotype 1 or 4 infection: a randomised study. *Gut*, *64*(6), 948-956.
21. Link, J. O., Taylor, J. G., Xu, L., Mitchell, M., Guo, H., Liu, H., ... & Desai, M. C. (2014). Discovery of ledipasvir (GS-5885): a potent, once-daily oral NS5A inhibitor for the treatment of hepatitis C virus infection. *Journal of medicinal chemistry*, *57*(5), 2033-2046.
22. De Ledinghen, V., & Vergniol, J. (2008). Transient elastography (fibroscan). *Gastroenterologie clinique et biologique*, *32*(6), 58-67.
23. Cheung, M. C., Walker, A. J., Hudson, B. E., Verma, S., McLauchlan, J., Mutimer, D. J., ... & UK, H. R. (2016). Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *Journal of hepatology*, *65*(4), 741-747.

24. Foster, G. R., Irving, W. L., Cheung, M. C., Walker, A. J., Hudson, B. E., Verma, S., ... & Hcv Research, U. K. (2016). Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *Journal of hepatology*, *64*(6), 1224-1231.
25. van der Meer, A. J., Maan, R., Veldt, B. J., Feld, J. J., Wedemeyer, H., Dufour, J. F., ... & Janssen, H. L. (2016). Improvement of platelets after SVR among patients with chronic HCV infection and advanced hepatic fibrosis. *Journal of gastroenterology and hepatology*, *31*(6), 1168-1176.
26. Knop, V., Hoppe, D., Welzel, T., Vermehren, J., Herrmann, E., Vermehren, A., ... & Welker, M. W. (2016). Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy. *Journal of viral hepatitis*, *23*(12), 994-1002.