Original Research Article

Changes of Liver Function Parameters in Hepatitis C Virus - Associated Compensated Liver Cirrhosis Patients Treated by Sofosbuvir/Daclatasvir +/- Ribavarin therapy. ~~A Retrospective Cohort Study~~

~~Abstract:~~

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INTRODUCTION :

Successful interferon free (IFN –free) treatment for hepatitis C patients are associated with improvement of liver function and blood parameters . To what extent of improvement do we see in compensated cirrhotics is unknown.

AIM:

To study liver function parameters in compensated cirrhotic HCV(Hepatitis C) patients receiving Sofosbuvir/Daclatasvir +/- Ribavarin therapy.

METHODOLOGY:

We here studied 55 consecutive patients with HCV associated liver cirrhosis including 43 patients with Child A cirrhosis and 12 patients with Child's B receiving combinations of direct acting antivirals Sofosbuvir/Daclatasvir with/without ribavirin that achieved sustained virological response at 12 weeks (SVR12) post treatment. The majority of patients was infected with HCV genotype 3 (n = 36); HCV genotypes 1a and 1b were present in 11 and 8 patients, respectively.

RESULTS

Parameters including Albumin , Prothrombin time , Hemoglobin showed no statistical significant difference post treatment in this study , however parameters of Platelet count, Bilirubin, Alanine Transferase and AFP all improved in the majority of patients during antiviral therapy irrespectively of

the underlying HCV genotype . For AFP , those with abnormal readings, 46.2% had reverted to a normal AFP after treatment. There was also an increase in platelet count from week 0-4 with the mean increase of 16.33 , then plateauing from weeks 4-24 weeks. For those with abnormal ALT at Week 0, 73.7% of them reverted to having normal ALT by week 24.

CONCLUSION

This real-world multi centre study showed that sofosbuvir /daclatasvir +/- ribavirin therapies in patients that have achieved SVR12 may indeed restore most liver and blood parameters in early compensated liver cirrhotics when HCV replication is successfully treated irrespective of the underlying HCV genotype. These improvements are maintained once treatment has ended . These findings are congruent with other real world studies that was done in decompensated cirrhotics receiving interferon free therapies .

Keywords : hepatitis c , sofosbuvir , ribavarin , cirrhosis

Background

Chronic infection with hepatitis C virus (HCV) affects approximately 130-150 million people worldwide and is a major cause of cirrhosis and hepatocellular carcinoma (HCC).[1,2]

For the past two decades chronic hepatitis C was treated with interferon alpha (IFNa) and ribavirin (RBV). IFNa/RBV combination therapy was associated with frequent and sometimes severe side effects.

The primary goal of HCV therapy is to cure the infection, i.e. to achieve a sustained virological response (SVR) defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion. An SVR corresponds to a cure of the HCV infection, with a very low chance of late relapse. Peginterferon alfa–ribavirin treatment for chronic HCV infection is associated with a sustained virologic response in approximately 40% of patients with genotype 1 infection and 75% of patients infected with genotype 2 or 3. [5 6]

In recent times we have moved away from peginteferon treatment and started direct acting antiviral (DAA) treatments for all HCV patients. Daclatasvir is a pangenotypic inhibitor of the non-structural NS5A protein of the HCV virus, and Sofosbuvir is a nucleotide analogue HCV NS5B polymerase inhibitor. [7, 8] SVR rates of daclatavir/ sofosbuvir antiviral with or without ribavirin regimens range from 86% - 97% depending on prior treatment history, genotype and

baseline cirrhosis of liver.[10-13]

From previous studies we have seen decompensated hepatitis C patients with variable other DAA regimes has been shown to be associated with improvement of liver function and may even lead to delisting of patients on the transplant waiting list.[9] However, it remains to be shown if suppression of viral replication would lead to similar clinical improvements in hepatitis C in compensated cirrhosis patients treated with Sof/ Dacla +/- Ribavarin DAA regime. It also remains to be shown the individual trend data of each blood parameter during this regime.

We here investigated the individual blood parameters consisting of ALB, BILI, AFP, HB, PLT, PT, ALT, MELD –NA SCORE in 55 consecutive patients with HCV-associated compensated liver cirrhosis including 78% with Child–Pugh A and 12% CPS B cirrhosis in a retrospective multicenter cohort study. The specific primary aims of the study is to determine to what extent impaired liver function in these patients may be restored by successful treatment of HCV by this DAA regime and to document trends of these individual blood parameters prior, during and after treatment.

Methodology

3.1 Study Type and Design

This is a retrospective multicenter cohort study including the first 55 chronic HCV patients with compensated liver cirrhosis receiving Sofosbuvir/Daclatasvir +/- ribavirin DAA therapy in the outpatient Gastroenterology + Hepatology clinic at Hospital Tengku Ampuan Rahimah Klang (HTAR), Malaysia and Hospital Raja Permaisuri Bainun Ipoh, Malaysia (HRPB) during the period of January 2018 - February 2020.

Data extraction and collection was done by assessing the individual patient card in the existing HCV registry of each center. A Hepatitis C registry was created prior to treatment of all HCV patients being treated with DAA Sofosbuvir/ Daclatasvir in each center. This registry was explored to select all patients fulfilling the criteria of this study. We then proceeded to evaluate the individual patient card to further extract results of individual's blood data parameters at week 0, week 4, week 12 and week 24.

All patients underwent ultrasound within the last 6 months before therapy. Liver cirrhosis was diagnosed either by definite clinical, biochemical,

ultrasonographic signs of cirrhosis and OGDS was done to assess portal hypertension complications. Only patients diagnosed with Child Pugh Score (CPS) A + B that have achieved sustained virologic response at 12 weeks (SVR 12) – defined as undetectable HCV RNA viral load 12 weeks after treatment completion were included in this study . No transient elastography was performed to assess liver fibrosis, as this modality was not available in either of these centers. Patients' blood data parameters was collected in the data collecting sheet at baseline week 0, week 4, week 12 and at week 24 of the antiviral therapy as these patients were seen on a 2-4weekly basis. Routine haematological and biochemical laboratory parameters were taken during the clinic visits and determined at the local laboratories. HCV RNA testing was performed with Xpert Cepheid HCV Viral load according to manufactures instructions with a lower limit of quantification and detection of 4.91 iu/mL. Routine individual parameters that were collected are Albumin, Bilirubin, Hemoglobin, Platelet, Prothrombin Time and Alanine Transferase (ALT). We then further extracted the parameters of alpha feto-protein (AFP), MELD –Na score prior to treatment and at the end of treatment only for comparison purposes as these parameters are not normally done as routine follow up.

The DAA used in this study is Sofosbuvir and Daclatasvir with or without Ribavirin. The full DAA regime decision and duration of treatment were prescribed according to the European Association for the Study of the Liver (EASL) [13] regime guidelines respectively. Treatment was usually scheduled for 12 - 24 weeks based on the guidelines above. Sofosbuvir was administered at 400mg (one tablet) once daily. Daclatasvir dose of 60 mg or 90 mg, once daily dose and an increased dose is needed for Retroviral Disease patients on Efavirenz drug for HIV.

Study Population

All Hepatitis C patients with CPS A + B that have undergone the above DAA antiviral regime and achieved SVR 12 from 1st January 2018 to 29th February 2020. Sample size was estimated between 50-60 patients. We have an average of 2-3 new patients being treated on a weekly basis at each center with Sofosbuvir/Daclatasvir DAA . This gives us a total estimate of 8 -10 from each centre or 16 - 20 from both centers on a monthly basis and out of this total , only 1/3rd of these patients being cirrhotic . Due to treatment duration is between 12- 24 weeks with a

further 12 wks. to confirm SVR results post treatment , all patients needed to have completed they're treatment regime by December 2019 and started latest by September 2019.

Statistical analysis

We planned to display our data as the following:
Continuous data (in mean (SD) for parametric data)
Categorical data is to be reported as whole numbers and in percentages
Comparison of 2 categories was done via chi-square and and comparison of 2 continuous variables was done via an independent t-test . All data analysis was entered and cleaned before analysis using SPSS v. 21.0. Any p value less than 0.05 was deemed significant.

Ethical clearance

Ethics was obtained from Malaysian Research Ethics Committee and registered on the National Medical Research Registry (NMRR-20-1345-54620). No identifiable were collected from the data obtained and all patients were analyzed anonymously.

Results

Demography of participants included in the study

The table below describes the demography of the participants included in the study. The mean age for participants were 57.45 (SD: 9.60). Most of the patients hailed from Ipoh (56.4%), were administered with ribavirin (69.1%), were of genotype 3 (65.5%), had no HIV (90.9%), of Child's Pugh Score A (78.2%)had no oesophageal varices upon presentation (58.2%)has no ascites during presentation (85.5%) and 78.2% did not undergo prior Hepatitis C treatment. There were equal males and females.

Table 1: Demography of participants included in the study

Variable	**n (%)** *N=55*
Age	57.45 (9.60)
Place data collected from	
Ipoh	31 (56.4)
Klang	24 (43.6)
Gender	
Male	28 (50.9)
Female	27 (49.1)
Administered with ribavirin	

Yes	38 (69.1)
No	17 (30.9)
Genotype	
1a	11 (20.0)
1b	8 (14.5)
3	36 (65.5)
Presence of HIV	
Yes	5 (9.1)
No	50 (90.9)
Child's Pugh score	
A	43 (78.2)
B	12 (21.8)
Presences of varices	
Yes	23 (41.8)
No	32 (58.2)
Presences of ascites	
Yes	7 (12.7)
No	48 (87.2)
Underwent prior Hepatitis C treatment	
Yes	11 (20.0)
No	44 (80.0)

Table 2 describes the measured variables of platelets, heamoglobin, ALT, bilirubin, AFP, albumin, HCVVL, PT and MELD-Na which is calculate as below.

MELD = 3.78×ln[serum bilirubin (mg/dL)] + 11.2×ln[INR] + 9.57×ln[serum creatinine (mg/dL)] + 6.43.
MELD-Na =MELD-Na-0.025 x MELD x (140-Na) +140

From the table we can determine that the highest platelets and PT were recorded in Week 4. Hemoglobin, ALT, Bilirubin, AFP and HCVVL were highest recorded at the baseline (Week 0). MELD and Albumin levels were highest recorded at Week 24. The full details are enclosed as below.

Table 2: Details of measured variables- platelet, hemoglobin, ALT, bilirubin, AFP, Albumin, HCVVL, PT and MELD

Variable	**Mean (SD)** *N=55*
Platelet	
Week 0	142.25 (73.99)
Week 4	153.89 (74.79)
Week 12	152.11 (80.88)
Week 24	138.68 (75.60)
Haemoglobin	
Week 0	13.18 (1.95)
Week 4	12.31 (1.95)
Week 12	12.74 (2.97)
Week 24	12.50 (1.90)
Alanine Transferase (ALT)	
Week 0	70.78 (53.30)
Week 4	35.53 (26.39)
Week 12	30.13 (20.00)
Week 24	32.18 (17.37)
Bilirubin	
Week 0	23.38 (17.38)
Week 4	22.76 (19.91)
Week 12	20.95 (18.59)
Week 24	20.55 (13.68)
Alpha Feto Protein (AFP)	
Week 0	12.15 (15.76)
Week 12	6.06 (4.46)
Week 24	8.69 (12.32)
Albumin	
Week 0	36.02 (6.96)
Week 4	35.98 (6.95)
Week 12	35.85 (7.71)
Week 24	36.18 (5.87)

Albumin (categorical data; *n (%)*) *Week 0* <35 ≥35 *Week 4* <35 ≥35 *Week 12* <35 ≥35 *Week 24* <35 ≥35	**N (%)** 25 (45.5) 30 (54.5) 20 (36.4) 35 (63.6) 19 (34.5) 36 (65.5) 15 (27.3) 23 (41.8)
Prothrombin Time (PT) *Week 0* *Week 4* *Week 12* *Week 24*	 15.02 (1.68) 23.10 (32.24) 14.50 (1.56) 15.74 (3.09)
Model of End Stage Liver Disease (MELD-Na) *Week 0* *Week 12* *Week 24*	 11.07 (4.22) 10.56 (4.76) 12.11 (5.14)

(Chi-squared testing of each parameter have been done separately @ bottom)
Demographics are being remained as descriptive stats

The table below describes the changes of platelets, hemoglobin, ALT, Bilirubin, AFP, Albumin, HCVVL, PT and MELD. From the results, it can be seen that there was an increase in platelets from week 0-4 with the mean increase of 16.33 (SD: 35.67), then dropping from weeks 4-12 then 12-24 weeks (plateauing at about -4). The comparison of the initial and end platelet results is about 0.69 (2.60) with the average change in weeks at a mean of 3.89 (16.80) For the hemoglobin, there was a general drop in values throughout the treatment length, however the mean of changes throughout the treatment was -0.21 (0.65). In the ALT status, it can be seen that the biggest mean change of all the weeks was from 0-24 weeks where there was a drop in the ALT status as much as -50.74 (51.10) .The average mean change over the weeks was also -17.37 (17.03). For the overall ALT change, we can see that the last observed ALT minus the first observed ALT yielded a change of -42.27 (46.98) with 80% of patients having a normal final ALT. For the bilirubin levels, the biggest drop

was seen at 0-24 weeks where patients generally had a reduction of -2.42 (10.56) in their bilirubin levels .The levels of AFP also had improvements with the biggest reduction coming from 0-4 weeks with a -5.67 (3.51). The overall change from the last AFP taken subtracted from the first AFP taken yielded a mean of -3.63 (8.19) with 76% of the patients having a normal last AFP reading. We did not observe significant changes in Albumin with the largest jump coming from 0-24 weeks at 1.47 (6.28). PT increase from 0-4 weeks and an overall change of the MELD score by 0.59 (4.82). Full details can be observed in the table below.

Table 3: The changes of values of variables collected at Week 0-4, 4-12, 12-24, 0-24 or otherwise specified

Variables		**Mean (SD)**
Platelets		
Change from		
	0-4 weeks	*16.33 (35.67)*
	4-12 weeks	*-4.51 (40.24)*
	12-24 weeks	*-4.03 (47.22)*
	0-24 weeks	*0.69 (2.60)*
	Average of changes over weeks	*3.89 (16.80)*
Hemoglobin		
Change from		
	0-4 weeks	*-0.86 (1.71)*
	4-12 weeks	*0.45 (3.14)*
	12-24 weeks	*-0.05(3.20)*
	0-24 weeks	*-0.68 (1.96)*
	Average of changes over weeks	*-0.21 (0.65)*
Alanine Transferase (ALT)		
Change from		
	0-4 weeks	-35.26 (48.60)
	4-12 weeks	-5.46 (19.02)
	12-24 weeks	-2.70 (11.81)
	0-24 weeks	-50.74 (51.10)
	Average of changes over weeks	-17.37 (17.03)
	Last ALT minus First ALT	-42.27 (46.98)
	Last ALT	***N (%)***
	Normal	44 (80.0)
	Abnormal	11 (20.0)
Bilirubin		
Change from		
	0-4 weeks	*-0.62 (15.84)*
	4-12 weeks	*-1.82 (5.20)*
	12-24 weeks	*-0.34 (7.33)*
	0-24 weeks	*-2.42 (10.56)*
	Average of changes over weeks	*-0.81 (3.52)*
Alpha feto protein (AFP)		
Change from		
	0-4 weeks	*-5.67 (3.51)*
	0-24 weeks	*-5.27 (7.27)*

	Average of changes over weeks	*-1.58 (8.21)*
	Last AFP minus first recorded AFP	*-3.63 (8.19)*
	Last AFP	***N (%)***
	Normal	*19 (76.0)*
	Abnormal	*6 (24.0)*
Albumin		
Change from		
	0-4 weeks	*-0.04 (4.85)*
	4-12 weeks	*-0.13 (4.33)*
	12-24 weeks	*1.39 (4.68)*
	0-24 weeks	*1.47 (6.28)*
	Average of changes over weeks	*0.49 (2.09)*
Prothrombin Time (PT)		
Change from		
	0-24 weeks	*7.93 (32.14)*
		0.69 (2.60)
Model of End Stage Liver Disease (MELD)		
Change from		
	0-24 weeks	*0.59 (4.82)*

AFP

Comparison of the first and last AFP done for patients

Table 4 describes the AFP change among patients (categorical form) from the pre-treatment to the last AFP taken (at 12 or 24 weeks). From the table we can see that those who started off with normal AFP had the constant reading through-out their therapy. For those with abnormal readings, 46.2% had reverted to a normal AFP after treatment. A chi square performed shows that there was a statistical significant difference (p=0.02) of change from pre to post. Eyeballing the data, it shows that there was a change of numbers from the abnormal to normal percentages.

Table 4: The comparison of alpha feto protein prior and after treatment

First AFP	**Last AFP n (%)**		***p* value**
	Normal	*Abnormal*	
Normal	*12 (100)*	*0*	**0.02**
Abnormal	*7 (53.8)*	*6 (46.2)*	

MELD

Comparison of the Week 0 and Week 24 MELD done for patients

Table 5 describes the MELD scores for patients at Week 0 and Week 24. The comparison of scores. An independent t-test down was done between the 2 mean scores. The test showed that there was no statistical significant difference (p=0.29) change in the MELD scoring signifying that the scores relatively remained the same.

Table 5: The comparison of the MELD score for patients in Week 0 and 24

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	11.07 (4.22)	54	0.29
24	12.11 (5.14)	55	

Albumin

Comparison of the albumin value changes at Week 4,12 and 24 compared to the baseline at Week 0

Graph 1 shows a scatter plot of all 55 patients' data (as much available in all weeks) according to their serum albumin levels. Table 6 shows details of the means (represented by lines in the graph), the sample sizes and the *p* value when comparing the means to the baseline mean (week 0). From the table and graph it can be seen that there was no statistical significant difference between the albumin levels when compared to the baseline reading.

Graph 1: A scatter plot displaying the levels of serum albumin according to Week 1,4,12 and 24 with the mean being displayed as a line

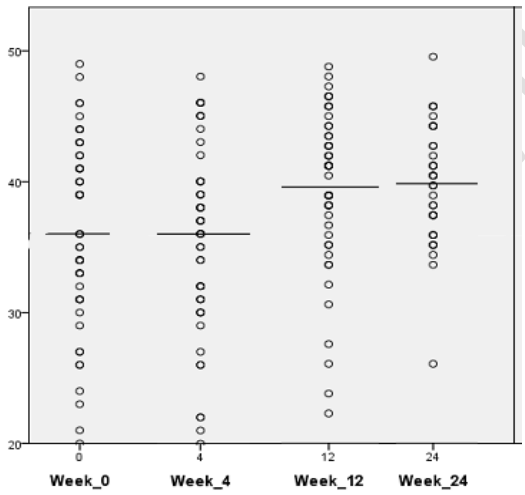

Table 6: Mean of serum albumin levels according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	36.02 (6.96)	55	-
4	35.98 (6.95)	55	0.98
12	35.85 (7.71)	55	0.90
24	36.18 (5.87)	38	0.91

PT

Comparison of the prothrombin value changes at Week 4,12 and 24 compared to the baseline at Week 0

Graph 2 and Table 7 shows the scatter plot of the PT according to the weeks and the mean, sample size and p values (comparing o the baseline at week 0) respectively. From the graph and tables, it can be seen that there was no statistical significant increase between the readings of the baseline (15.02 [1.68]) and week 4 (23.10 [32.24]) which yielded a *p* value of 0.06. Week 12 and week 24 had no statistical significant difference when compared to the baseline showing no effects on PT of our patients while on DAA therapy.

Graph 2: A scatter plot displaying the levels of PT according to Week 1,4,12 and 24 with the mean being displayed as a line

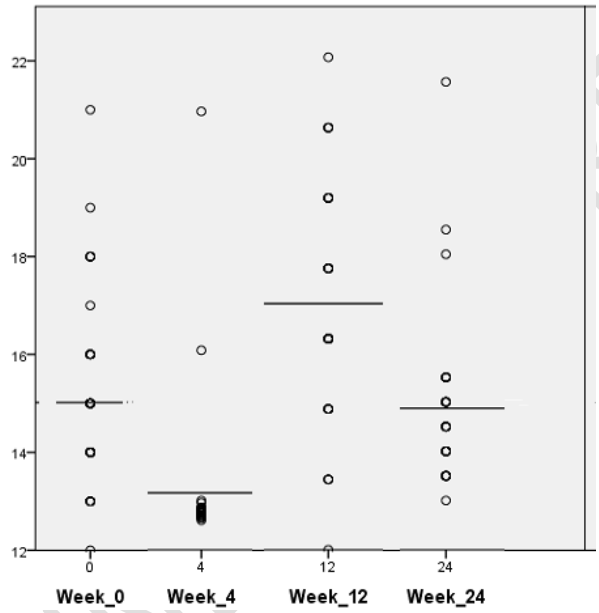

Table 7: Mean of PT levels according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	15.02 (1.68)	54	-
4	23.10 (32.24)	15	**0.06***
12	14.50 (1.56)	15	0.17
24	15.74 (3.09)	13	0.15

Albumin < 35

Comparison of the patients with albumin <35 value changes at Week 4,12 and 24 compared to the baseline at Week 0

The graph and table below represents the level of serum albumin below 35 among the patients affected (with serum albumin below 35) according to weeks and the mean, sample size and p value (comparison with baseline at week 0) respectively. From the data and the table it can be seen that there was no statistical significant difference among the levels of patients having serum albumin <35 measured at the intervals. Clinically we see no improvement but a maintenance in albumin of our patients during DAA therapy for this group.

Graph 3: A scatter plot displaying the levels of patients with serum albumin <35 according to Week 1,4,12 and 24 with the mean being displayed as a line

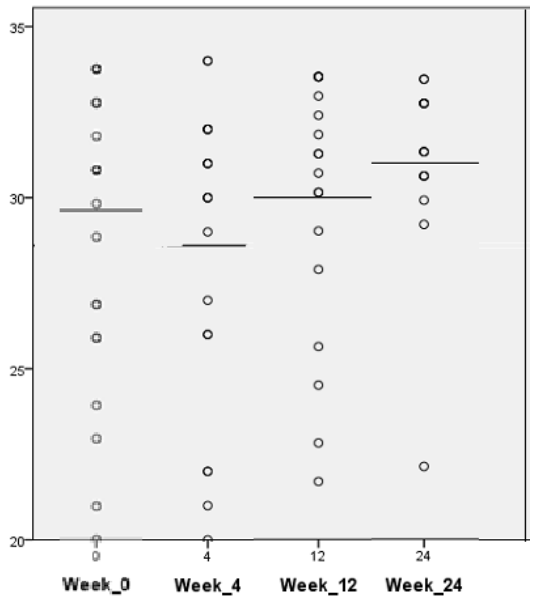

Table 8: Mean of patients with serum albumin <35 according to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	29.80 (4.36)	25	-
4	28.60 (4.37)	20	0.36
12	27.74 (6.73)	19	0.22
24	30.53 (3.93)	15	0.60

ALT >40

The graph below shows the distribution of the patients of having ALT>40 according to weeks. The mean for week 0 was 70.79 (53.30) among those with the ALT. The mean of the ALT drop to normal values from Week 4 onwards forming a near plateau until Week 24. An independent t-test to test the statistical significant difference between each of Week 4, 12 and 24 with Week 0 showed a statistical significant difference (p<0.001). This shows that the reduction of ALT in each week compared to Week 0 had a statistical significant difference in reduction. We see ALT values normalised by week 4 and were maintained at follow-up week 12 and subsequently.

Graph 4: A scatter plot displaying the levels of patients with ALT>40 according to Week 1,4,12 and 24 with the mean being displayed as a line

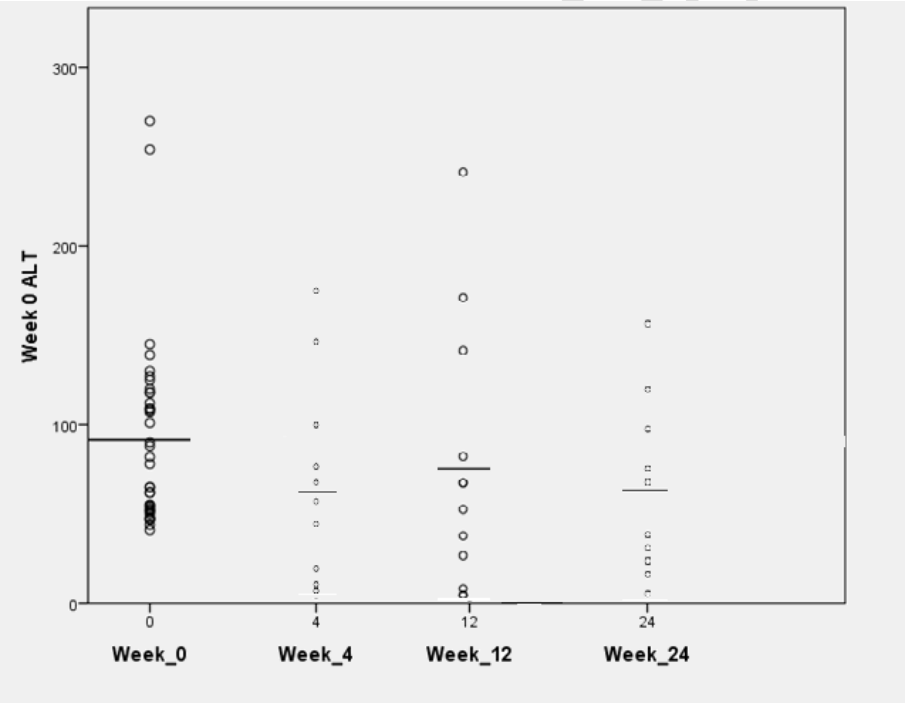

Table 10: Mean of patients with ALT to the weeks. *p* value comparison done with baseline

Week	Mean (SD)	Sample	*p* value compared with Week 0
0	70.78 (53.30)	55	-
4	35.53 (26.39)	55	<0.001
12	30.13 (20.00)	54	<0.001
24	32.18 (17.37)	38	<0.001

DISCUSSION

Successful antiviral treatment of compensated hepatitis C with Sofosbuvir/ Daclatasvir +/- Ribavarin is associated with improvement of liver function that sustains even after treatment. We here show parameter changes of liver function test at week 0,4,12 and 24 in a retrospective multi center cohort of consecutive patients with compensated HCV cirrhosis on treatment . We further show (i) liver function parameters may recover in the majority of patients when HCV replication is successfully blocked irrespectively of the underlying HCV genotype, (ii) that these improvements are maintained even after treatment is stopped.

We here note rapid improvement of some of the liver function parameters already within the first 2–4 weeks of therapy. In particular serum ALT levels improved fast during treatment in patients. Liver enzymes also quickly normalize during interferon-free therapy of hepatitis C indicating reduced hepatic inflammation.[15] It has recently been shown that viral clearance is accompanied by a rapid down regulation of various intrahepatic IFN- stimulated genes.[16] Combining these and our findings support the concept that intrahepatic inflammation directly contributes to reduced synthesizing capacity of the liver and that blocking inflammation can restore liver function to some extent. However, not all parameters showed an early improvement during therapy but only changed until the end of treatment or even during follow-up. These parameters included AFP and bilirubin. It is also known for more than 20 years that platelet counts increase during ribavirin therapy[17, 18] and there- fore on-treatment platelet increases may not necessarily indicate improvements in portal hypertension. Further studies are needed to prove if these improvements are solely due to ribavirin effect .

In conclusion, the present study shows changes in blood parameters for patients consuming HCV treatment above on a complete scheduled follow up manner and shows post treatment parameter changes . It is likely that hepatic function may at least partially be restored in the majority of patients if HCV RNA replication is blocked – potentially reducing the need for liver transplantations. However, further follow-up is needed and patients should be screened in particular for the development of HCC.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. There is not any conflict of interest between the authors and producers of the products used in this research. Also, the research was not funded by the product company.

References

1. European Union HCV Collaborators. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. Lancet Gastroenterol Hepatol 2017;2:325–336.

2. World Health Organization. Global alert and response (GAR). Hepatitis C. 2012 (http://www.who.int/csr/disease/hepatitis/ whocdscsrlyo2003/en/index4.html).

3. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut 2006; 55: 1350–9.

4. S[1]ulkowski 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

5. McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580-93. [Erratum, N Engl J Med 2009;361:1027.]

6. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-55.

7.van der Meer AJ, Wedemeyer H, Feld JJ, et al. Life expectancy in patients with chronic HCV infection and cirrhosis compared with a general population. JAMA 2014; 312:

8.1927–8. Perrillo RP, Wright T, Rakela J, et al. A multicenter United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. Hepatology 2001; 33: 424–32.

9. K. Deterding, C. Ho€ner zu Siederdissen, K. Port, P. Solbach, L. Sollik, J. Kirschner, C. Mix, J. Cornberg, D. Worzala, H. Mix, M. P. Manns, M. Cornberg[a] & H. Wedemeyer : Improvement of liver function parameters in advanced HCV-associated liver cirrhosis by IFN-free antiviral therapies

10. David R. Nelson James N. Cooper Jacob P. Lalezari Eric Lawitz Paul J. Pockros Norman Gitlin Bradley F. Freilich Ziad H. Younes William Harlan Reem Ghalib Godson Oguchi Paul J. Thuluvath Grisell Ortiz- Lasanta Mordechai Rabinovitz David Bernstein Michael Bennett Trevor Hawkins Natarajan Ravendhran Aasim M. Sheikh Peter Varunok Kris V. Kowdley Delphine Hennicken Fiona McPhee Khurram Rana Eric A. Hughes. All- oral 12- week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY- 3 phase III study

11. Vincent Leroy Peter Angus Jean- Pierre Bronowicki Gregory J. Dore Christophe Hezode Stephen Pianko Stanislas Pol Katherine Stuart Edmund Tse Fiona McPhee Rafia Bhore Maria Jesus Jimenez- Exposito Alexander J. Thompson. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY- 3+)

12. Anne F. Luetkemeyer, Cheryl McDonald, Moti Ramgopal, Stephanie Noviello, Rafia Bhore, Peter Ackerman . 12 Weeks of Daclatasvir in Combination With Sofosbuvir for HIV-HCV Coinfection (ALLY-2 Study): Efficacy and Safety by HIV Combination Antiretroviral Regimens

13. Poordad F, Schiff ER, Vierling JM, et al. Daclatasvir, sofosbuvir, and ribavirin combination for HCV patients with advanced cirrhosis or postransplant recurrence: phase 3 ALLY-1 study. 50th Annual Meeting of the European Association for the Study of the Liver (EASL); April 22-16, 2015; Vienna, Austria.

14. EASL recommendation on treatment of hepatitic C 2016 :https://easl.eu/wp-content/uploads/2016/10/Summary-HCV-2016.pdf

15. Osinusi A, Meissner EG, Lee YJ, Bon D, Heytens L, Nelson A, Sneller M, Kohli A, Barrett L, Proschan M, Herrmann E. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. Jama. 2013 Aug 28;310(8):804-11.

16. Meissner EG, Wu D, Osinusi A, Bon D, Virtaneva K, Sturdevant D, Porcella S, Wang H, Herrmann E, McHutchison J, Suffredini AF. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. The Journal of clinical investigation. 2014 Aug 1;124(8):3352-63.

17. Mihm U, Welker MW, Teuber G, Wedemeyer H, Berg T, Sarrazin C, Böhm S, Alshuth U, Herrmann E, Zeuzem S. Impact of ribavirin priming on viral kinetics and treatment response in chronic hepatitis C genotype 1 infection. Journal of viral hepatitis. 2014 Jan;21(1):42-52.

18.di Bisceglie AM, Shindo M, Fong TL, Fried MW, Swain MG, Bergasa NV, Axiotis CA, Waggoner JG, Park Y, Hoofnagle JH. A pilot study of ribavirin therapy for chronic hepatitis C. Hepatology. 1992 Sep;16(3):649-54.