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Time-dependent improvement of liver inflammation, fibrosis and metabolic liver function after successful direct-acting antiviral therapy of chronic hepatitis C

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Time-dependent improvement of liver inflammation, fibrosis, and metabolic liver function after successful direct-acting antiviral therapy of chronic hepatitis C

Running title: Time-dependent effects of DAA-therapy

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Abstract (250 words)

Sofosbuvir-based direct-acting antiviral (DAA)-therapy generally cures chronic hepatitis C (CHC) infections, however, the effects on the underlying liver disease and the potential rate of recovery are unclear. We aimed to investigate the effects of DAA-therapy on liver inflammation, fibrosis, metabolic function and cognitive function and the time course in CHC patients with advanced liver disease. Seventy-one CHC patients with advanced liver disease were studied before, during, and one year after successful sofosbuvir-based DAA-therapy. Liver inflammation was assessed by plasma sCD163 and sMR levels (ELISA), fibrosis by liver stiffness (transient elastography), function by galactose elimination capacity (GEC), and cognitive performance by continuous reaction time (CRT).

During DAA-therapy, we observed a rapid sCD163 decline from baseline to end of treatment (EOT) (6.9 vs. 3.8 mg/L, $p < 0.0001$), whereas the change in sMR was more subtle (0.37 vs. 0.30 mg/L, $p < 0.0001$). Liver stiffness decreased by 20% at end of treatment (17.8 vs. 14.3 kPa, $p < 0.0001$), together suggesting rapid resolution of liver inflammation. One-year after treatment, liver stiffness decreased by an additional 15% ($p < 0.0001$), suggestive of fibrosis regression. The GEC improved at follow-up (all: 1.74 vs. 1.98 mmol/min), mainly at 12-weeks post-treatment, both in patients with cirrhosis (n=56) and those with advanced liver fibrosis (n=15) ($p < 0.001$). The CRT improved at one-year follow-up (1.86 vs. 2.09, $p = 0.04$). In conclusion,

successful DAA-therapy of CHC proves beneficial in advanced liver disease, with an initial rapid resolution of liver inflammation and a subsequent gradual but steady improvement in liver fibrosis, metabolic liver function, and reaction time.

Introduction

In patients with chronic hepatitis C (CHC), the viral infection is a constant trigger of inflammation, which subsequently induces formation of fibrosis. With viral clearance, the inflammation ceases and thus, the inducer of fibrosis disappears. Patients with CHC are at risk of developing cirrhosis and subsequent complications related to portal hypertension and hepatocellular carcinoma (HCC) resulting in increased morbidity and mortality [1]. These risks are consequences of the pathological processes in the liver, i.e. inflammation and progression of fibrosis with the subsequent loss of metabolic liver function.

The all-oral interferon-free direct-acting antiviral (DAA) therapies cure patients with hepatitis C virus (HCV) in the majority of cases. Sofosbuvir is a DAA with pan-genotypic effects, which in combination with other DAAs or ribavirin provides high sustained virologic response (SVR) rates for CHC patients including those with advanced liver disease [2,3]. The effect of DAA-treatment on the underlying liver disease and associated complications is crucial for the prognosis and future management of the patients, but evidence is lacking. Some studies have reported decreased liver stiffness after DAA-therapy and viral clearance [4,5], while another reported slight changes in portal hypertension [6]. Except from HCC study results [7-11], few studies have assessed long-term hepatic disease progression [12,13] or mortality after SVR [14], and none have assessed the metabolic liver function after DAA-therapy. Thus, it remains unclear in which order the potential changes occur, how quickly the changes occur, whether liver inflammation, liver fibrosis, and liver function are all affected and how these parameters associate internally after DAA-therapy.

Liver inflammation reflected by macrophage activation may be assessed by measurement of the circulating macrophage activation markers, soluble (s) CD163 and the soluble mannose receptor (sMR), which are elevated in parallel with severity in chronic liver diseases [15-18]. Liver fibrosis is non-invasively assessed by measurement of liver stiffness using methods such as transient elastography (TE) by FibroScan or acoustic radiation force impulse (ARFI) scans. Liver stiffness is influenced by a dynamic inflammatory component and structural components, why it provides insight into both liver inflammation and fibrosis [19]. Liver function may be assessed by the use of standard blood parameters; however, this approach provides only limited understanding of the complex functions of the liver. Other potential measures of liver function are the galactose elimination capacity (GEC) test, which quantifies the metabolism of galactose by galactokinase in the hepatocyte cytoplasm, thus reflecting functional liver cell mass [20]. Moreover, the continuous

reaction time (CRT) reflects cognitive function in patient with severe liver disease and potentially the degree of hepatic encephalopathy (HE) [21].

Thus, we aimed to investigate the effect of sofosbuvir-based DAA-therapy on macrophage activation, liver stiffness, metabolic liver function, and reaction time in CHC patients with advanced liver disease. We hypothesised that sofosbuvir-based DAA-therapy time-dependently improves liver inflammation, fibrosis, and function with the majority of the initial effect being on inflammation, while the longer-term effects pertain to fibrosis and liver function improvements.

Materials and Methods

Patients and design

A Danish multi-centre, prospective cohort study was performed among CHC patients undergoing sofosbuvir-based DAA-therapy. The patients were included between September 2014 and February 2017 in the Central Denmark Region at the Department of Hepatology and Gastroenterology and at the Department of Infectious Diseases at Aarhus University Hospital, Aarhus, as well as at the Diagnostic Centre, Silkeborg Regional Hospital, Silkeborg, and the Department of Medicine, Viborg Regional Hospital, Viborg, where they were referred for evaluation and treatment of CHC. Inclusion criteria were CHC with severe liver disease (METAVIR F≥3 or liver stiffness>10 kPa), age ≥18 years, and planned initiation of sofosbuvir-based DAA-therapy. A diagnosis of cirrhosis was based on either liver biopsy, or a combination of liver stiffness>15 kPa [22] with radiological, clinical, and/or biochemical signs of liver cirrhosis. The cirrhosis patients were routinely screened for HCC with biannual ultrasound scans. Exclusion criteria were co-infection with HBV or HIV and excessive alcohol intake within the last 6 months preceding treatment initiation. In total, 143 patients with CHC were screened for participation in the study and 71 (50%) patients were included 13 treatment-experienced; 9 of these had failed interferon-based therapies and 4 DAA-treatments as well. The reasons for screening failure of the 72 patients who were not included in the study are shown in Supplementary figure 1. Interim results on macrophage activation and liver stiffness from the first 38 patients have previously been reported in another study from our group [23]. The study was performed in accordance with the Helsinki declaration and the ethical review board in the Central Denmark Region approved the study (1-10-72-199-14). All included patients signed informed consent forms.

DAA-therapy

The decisions on whether to initiate treatment and the treatment regimen were made in consensus by a team of experienced clinicians according to national guidelines at the time of the study. The treatment regimens included sofosbuvir and potentially a second DAA, i.e. daclatasvir, simeprevir, or ledipasvir, depending on the genotype. Some regimens also included weight-based ribavirin with potential dose reduction at the occurrence of anaemia. For treatment details see Supplementary table 1. Most patients were treated for 12 weeks, however extension of treatment to 16 or 24 weeks was made at the discretion of the responsible physicians. Viral load measurements were obtained at all visits and determined using qPCR assays with a lower

detection limit of 9 IU/mL and a lower quantifiable limit of 15 IU/mL. SVR was defined as undetectable HCV-RNA at 12 weeks (SVR12) and one year (SVR52) after treatment cessation. Adverse events (AE) and serious adverse events (SAE) were recorded and SAEs were reported to relevant authorities. In total, 73% of the patients experienced adverse events during treatment. In general, these were mild and mainly present during the first treatment week (Supplementary table 2).

Biochemical methods

Blood sampling was performed before treatment, at week 1, 2, 4, and 8 during treatment, at the end of treatment (EOT), and 12 weeks and one year after treatment (Supplementary figure 1). If the treatment period was extended, blood sampling was performed every 4 weeks during the extension. Standard biochemical parameters were analysed using validated assays at the respective sites. The Child-Pugh score was calculated at each visit using bilirubin, albumin and international normalized ratio (INR), as well as the presence of HE and ascites. The model of end-stage liver disease (MELD) score was calculated using bilirubin, creatinine, and INR. The quantification of sCD163 and sMR was performed by use of an in-house sandwich enzyme-linked immunosorbent assays (ELISA) using a BEP-2000 ELISA-analyser (Dade Behring, Siemens, Erlangen, Germany) as previously described [16,24]. sCD163 and sMR are stable at -80°C for long periods of time and resistant to repeated freezing and thawing. Control samples and standards were included in each run.

Measures of liver stiffness and portal hypertension

Liver stiffness was assessed using transient elastography (FibroScan, Echosens, Paris, France) or ARFI-scans (Siemens, Erlangen, Germany). The measurements were performed by trained doctors, nurses or bio technicians before initiation of treatment, at EOT, at 12 weeks and one-year post-treatment. To ensure the validity of the FibroScan measurements, at least 60% valid measurements in a complete series (10 approved measurements), had to be achieved. Additionally, the interquartile range (IQR) had to be below 25% of the median for the result to be approved as recommend in the Central Denmark Region. The ARFI-elastography is integrated into a conventional ultrasound device and was performed as previously described [25]. The liver stiffness data are reported separately for the FibroScan and the ARFI-scans and as a percentage, where all

baseline values are set as 100% and the subsequent values are reported as percentage of the baseline value.

In patients with a clinical indication, liver vein catheterizations were performed to evaluate the degree of portal hypertension using an established method as previously described, thus estimating the hepatic venous pressure gradient (HVPG) [26].

Metabolic liver function by the galactose eliminations capacity (GEC) test

Galactose is almost exclusively metabolized in the liver, and the GEC evaluates the ability of the liver to eliminate galactose from the bloodstream, thus, it may be used as a quantitative measure of the metabolic capacity of the liver [27]. Briefly explained, a galactose solution is injected intravenously over 5 minutes (1 ml/kg body weight of a 500 mg/mL galactose solution), arterialized capillary blood is sampled 8 times every 5 minutes from 20 minutes after the injection to measure the blood concentration of galactose, and urine is collected for 4 hours to measure urinary excretion of galactose. The GEC is calculated from the injected amount subtracting the excreted amount and from the straight line connecting the observations of the blood galactose concentration against time [28]. For each patient, GEC results are presented as millimoles galactose cleared per minute (mmol/min) and a percentage of an expected normal value based on weight (%).

Continuous reaction time (CRT)

The CRT evaluates cerebral function by testing the ability to react to sounds for a longer period of time. The test is performed using a laptop, dedicated software (EKHO, Bitmatic, Aarhus, Denmark), headphones, and a trigger button as previously described [21]. Results are reported as the variation coefficient of the reaction times delivered during the test called the CRT index, which reflects reaction time stability. A CRT index below 1.9 is considered pathological, and may reflect minimal HE in liver disease [21].

Statistical methods

Changes over time were evaluated with univariate or multivariate repeated measurements analysis of variance (ANOVA) using mixed models. Equality or inequality of standard deviations and correlations in the models were considered as appropriate. Model validation was performed by comparing observed and expected within subject standard deviations and correlations by

inspecting QQ-plots. Student's t-tests were used to study differences between groups, and one-way ANOVAs were used for the comparisons of multiple groups. Log-transformation was performed when appropriate. For the non-normally distributed data, Kruskal-Wallis and Mann-Whitney tests were used. The relationships between continuous variables were analysed using Spearman's rank correlation. Binary data were analysed using Chi^2 or Fisher's exact depending on sample size. All data are reported as medians with 95% confidence intervals (CI) or proportions unless otherwise stated. P-values <0.05 are considered statistically significant. The data analyses were performed using STATA v. 14.0 (Stata Corp LP, College Station, TX, USA).

Results

Baseline patient characteristics

In total, 71 patients with CHC were included in the study. Baseline patient characteristics are shown in Table 1. All the patients, both treatment-naïve and -experienced, achieved SVR12 and SVR52 except for one treatment-experienced patient initially infected with genotype 1, but re-infected with a genotype 3-strain 12 weeks after treatment. Overall, this corresponds to an SVR-rate of 98.6%. At baseline, all patients had advanced liver disease with inflammation corresponding to elevated alanine aminotransferase (ALT) levels [77 IU/L (95% CI:66-87)] and elevation of the sCD163 level to nearly twice the upper normal limit [6.9 mg/L (6.0-7.8), reference interval: 0.7-3.9 mg/L]. For sMR, the baseline level was 0.37 mg/L (0.34-0.42) with a reference interval of 0.10-0.43 mg/L. The macrophage activation markers correlated with each other and a number of biochemical parameters, most notably albumin, alkaline phosphatase, bilirubin and α-fetoprotein (Supplementary table 3).

Of the 71 included patients, 15 (21%) patients had advanced liver fibrosis and 56 (79%) patients had cirrhosis; 49 with Child-Pugh A cirrhosis and 7 with Child-Pugh B cirrhosis. The median liver stiffness was 22 kPa (18-31) using FibroScan (n=43) and 1.9 m/s (1.8-2.0) on the ARFI-scans (n=28). Liver stiffness was highest in the Child-Pugh B patients [40.0 kPa (25.5-75), 2.6 m/s (1.9-3.3)]. The median MELD-score was 8.5 (7.5-8.5) for the entire group and higher in the Child-Pugh B patients [11.7 (8.9-14.0)]. The GEC was performed in 54 (76%) patients before treatment with a median value of 1.74 (1.60-1.88), corresponding to 59% (56-64) of normal. The patients with cirrhosis had lower metabolic liver function by GEC at baseline compared with the patients who had advanced liver fibrosis (57% vs. 76%, p=0.01). At baseline, 44 (62%) patients had a CRT

performed with a mean CRT index of 1.86 (1.66-2.06), which is below the normal limit of 1.9 suggestive of minimal HE.

Effects of DAA-therapy on liver inflammation and fibrosis

ALT rapidly improved with a 50% decrease during the first week of DAA-therapy and normalization by EOT (Table 2). The levels of sCD163 and sMR decreased by 44% and 19%, respectively, from baseline to EOT (p<0.0001), suggesting rapid resolution of liver inflammation (Figure 1). There was a significant increase (17%) in sCD163 from EOT to 12-weeks post-treatment followed by a similar significant decrease (14%) at one-year post-treatment (p<0.0001). There were no changes in sMR at EOT compared with the levels at follow-up (p>0.3, both). The patients with no history of excessive alcohol consumption had a significantly more pronounced decrease in sCD163 during the study period (p<0.001); this was not the case for sMR (p=0.19).

Over the entire study period, there was a significant decrease in liver stiffness as the relative change from baseline (p<0.001). A large part of the decrease occurred before EOT; thus, the liver stiffness decreased by 14% at EOT (8-20, p<0.0001) (Figure 2). From EOT to one-year post-treatment, liver stiffness decreased by a further 11% (5-18) and by 6% (1-13%) between 12-weeks and one-year post-treatment. Thus, in the patients with advanced fibrosis, the liver stiffness reached a final median value at one-year post-treatment of 5.5 kPa (4.1-8.6) on FibroScans and 1.39 m/s (1.22-1.70) on the ARFI-scans. The cirrhosis patients ended up at a median liver stiffness of 13.5 kPa/1.81 m/s (10.2-20.5 kPa/ 1.70-2.10 m/s). For the Child-Pugh B patients the median liver stiffness ended at 39.8 kPa/ 3.16 m/s (14.8-72 / 2.37-3.95 m/s) at one-year post-treatment follow-up. The decrease in liver stiffness was accompanied by a similar significant increase in albumin from EOT to one-year post-treatment (35.7 (34.5-36.8) vs. 37.5 (36.2-38.7), p<0.001), and was complemented by an overall half point decrease in mean MELD score (8.6 (8.0-9.2) vs. 8.1 (7.5-8.7), p=0.03).

Effects of DAA-therapy on liver function

Thirty-six (51%) patients completed a follow-up GEC and among those the outcome of the test improved significantly over the entire study period (p<0.001) (Figure 3). The increase was significant from baseline compared with follow-up both at 12-weeks and one-year post-treatment (p=0.001), however with no significant change between 12-weeks and one-year post-treatment

(p=0.71). At one-year post-treatment, the mean GEC for the entire cohort was improved to 1.98 mmol/min (1.83-2.14), which corresponded to 66% (61-72) of normal (n=26) compared with 59% (56-64) at baseline. The difference in metabolic liver function between cirrhosis patients and advanced fibrosis patients was sustained during the entire study period with the cirrhosis patients ending at 1.95 mmol/min (1.80-2.10) (64%) and the patients with advanced fibrosis at 2.24 mmol/min (1.88-2.60) (82%) (Figure 3).

At 12-weeks post-treatment, 36 (51%) patients completed a follow-up CRT providing a mean CRT index of 2.03 (1.82-2.24). One-year after treatment cessation, the mean CRT index was 2.09 (1.87-2.32) (n=29), which corresponded to an increase of 0.24 (0.01-0.47) in the CRT index from baseline to one-year after treatment (p=0.04) (Figure 4).

Correlations between liver inflammation, fibrosis, and function before and after DAA-therapy

The levels of sCD163 and sMR were more than 40% higher in the 56 patients with cirrhosis at all times points in comparison with the 15 patients with advanced liver fibrosis (50% (14-98%) and 40% (11-76%), respectively (p<0.01). Five cirrhosis patients (7%) developed *de novo* HCC and two patients recurrent HCC during follow-up (3%). In a mixed model, the dynamics of sCD163 were different over time in the patients who developed HCC compared with the patients who did not (p=0.02), with tendencies of increased levels at EOT and during follow-up in HCC patients (sCD163: p<0.16, sMR: p<0.07). The levels of the macrophage activation markers correlated with liver stiffness at baseline (r>0.39, p<0.05) (Supplementary table 3), and at follow-up, though only when using the FibroScans (r>0.48, p<0.03). At baseline, we found inverse correlations between the macrophage activation markers and the GEC (r=-0.41/-0.47, p<0.003) (Supplementary table 3) but no association at follow-up (p>0.08). The levels of sCD163 and sMR were not significantly associated with CRT at baseline (Supplementary table 3) or during follow-up, except for a negative correlation between sMR and the CRT 12-weeks after treatment (r=-0.43, p=0.02). The changes in sCD163 or sMR did not correlate with the changes in liver stiffness, GEC, or CRT neither during or after treatment (p>0.07).

Discussion

This study demonstrates the time-dependent liver-related effects of successful sofosbuvir-based DAA-therapy in patients with CHC and advanced liver disease, the majority with cirrhosis. The main effect on liver inflammation is rapid and instigates early after therapy initiation. The long-term effects consist of diminished liver stiffness and improved metabolic liver function and reaction time. Interestingly, the effects on liver function occur as early as 12 weeks after treatment and are sustained at one-year follow-up.

The major strength of the study is the prospective design and comprehensive work-up of the patients. We included all consecutively consenting patients with advanced liver disease or cirrhosis planned to initiate sofosbuvir-based DAA-therapy at hospitals in the Central Denmark Region over a two and a half-year period, hence providing generalizable results from a real-life cohort. The limitations of the study, though, pertain to the lack of histological verification of liver inflammation and fibrosis. However, we and others have previously shown very good associations between the macrophage activation markers and liver inflammation [15,23] as well as between liver stiffness and fibrosis, even after SVR [29,30]. Another limitation is that not all patients underwent transient elastography by the same method being either the FibroScan or the ARFI-method, however as the combined and separate associations seem similar, we consider the results valid. However, it is interesting that the magnitude of the changes seem larger for the FibroScan results compared with the ARFI-scan results and it might be suggested that the outcome of FibroScan may be more affected by the presence of liver inflammation.

As part of the inflammatory processes in the liver, CD163 and the mannose receptor (MR) are released from the surface of activated macrophages in the liver and are found in the blood as circulating sCD163 and sMR [16,24]. The receptors are differentially expressed on different macrophage subtypes [31,32]. In addition, CD163 is a lineage specific monocyte/macrophage marker, while the MR is also detected on the surface of dendritic and endothelial cells. The CD163 receptor is known to specifically bind hemoglobin-haptoglobin complexes, whereas the MR binds a variety of endogenous and exogenous substances, why their mechanisms of shedding are probably different [33,34]. This may explain the difference in the degree of elevation between sCD163 and sMR in CHC patients, as well as the different dynamics of the two markers during and after DAA-treatment.

The observations of rapidly improved ALT and sCD163 values combined with a decrease in liver stiffness within the period of DAA-therapy suggest prompt resolution of liver inflammation in

parallel with clearance of the hepatitis C virus. This is supported by several studies with similar results regarding inflammatory mediators, sCD163 and liver stiffness during DAA-therapy [35-37].

Next, we examined the long-term effects of DAA-therapy on liver fibrosis during one-year follow-up after treatment and found a continued decrease in liver stiffness. Whereas ALT and sCD163 levels had plateaued already at EOT, liver stiffness continued to decrease possibly because of subsequent regression of liver fibrosis. Fibrosis regression has been investigated extensively in chronic hepatitis B patients after nucleoside treatment and in CHC patients after interferon treatments with results pointing towards a potential for regression or even resolution at longer follow-up [38,39]. A similar effect of DAA-therapies is becoming evident and fibrosis regression may also lead to diminished portal hypertension with discrete improvements in portal pressure after DAA-therapy [6,30]. In a small number of patients (n=4), we observed a trend towards reduced portal hypertension. This was accompanied by improved platelet count, albumin levels and MELD scores, suggestive of improvement of liver disease severity related to diminished fibrosis.

The improvements in liver inflammation and fibrosis are likely the reason for the augmented metabolic liver function, as others have shown that the GEC is associated with liver inflammation, disease severity, and prognosis in cirrhosis patients [28,40,41]. Thus, we highlight the improved metabolic liver function as an important clinical finding.

Many cirrhosis patients have minimal HE with decreased quality of life and increased risk of developing clinically manifest HE [42]. We assessed the cognitive function of our patients by quantifying reaction time stability using the CRT. At baseline, the patients indeed had a mean CRT index within the pathological limits suggestive of minimal HE. However, with DAA-therapy, this improved and the mean level ended above the cut-off level. The change of 0.24 is between the values of what is observed with the usual recommended anti-HE medication and placebo in cirrhosis patients [43], and may potentially be attributed to reduced cerebral inflammation and improved neural health [44,45]. The improvements in GEC and reaction time is promising and calls for further studies with longer follow-up to assess the potential improved prognosis for patients with CHC and advanced liver disease or cirrhosis undergoing DAA-treatment.

Even though the overall results are generally positive, several patients deteriorated after DAA-therapy. One cirrhosis patient decompensated with acute variceal bleeding, five cirrhosis patients developed *de novo* HCC and two patients recurrent HCC. The sCD163 levels correlated with alpha-fetoprotein and the sCD163 dynamics over the entire study period was different between

patients with HCC compared with patients without HCC, which may suggest that sCD163 may be valuable in the surveillance of patients after SVR.

Taken together, this study shows that with successful DAA-therapy a rapid resolution of liver inflammation occurs followed by more subtle changes in liver fibrosis. Further, this study is the first to show the beneficial effects of DAA-therapy on metabolic liver function and reaction time in patients with CHC and advanced liver disease, thus, suggesting a possibility for improved outcome with perspectives such as decreased morbidity and mortality.

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Tables

Table 1. Baseline patient characteristics.

	Number (%)	Median (95% CI)
Age (Years)		55 (53-58)
Gender		
Male	45 (63)	
Female	26 (37)	
BMI (kg/m^2)		25 (24-27)
Diabetes	11 (15)	
Alcohol		
No alcohol †	31 (44)	
Occasional	1 (1)	
Previous over intake	39 (55)	
Genotype		
1a	14 (20)	
1b	3 (4)	
2	2 (3)	
2b	1 (1)	
3	36 (51)	
3a	14 (20)	
6e	1 (1)	
Ethnicity		
Caucasian	67 (94)	
Other	4 (6)	
MELD score		8.5 (7.5-8.5)
Liver stiffness		
Fibroscan (kPa) (n=43)		22 (18-31)
ARFI-scan (m/s) (n=28)		1.9 (1.8-2.0)
Cirrhosis	56 (79)	
Child-Pugh Score		5 (5-6)
Child-Pugh Class		

	A	49 (69)	
	B	7 (10)	
Previous HCC		4 (6)	
Alpha-fetoprotein (x10^3 IU/L)			5 (4-5)
Gastroscopy		54 (76)	
	No varices	40 (56)	
	Grade 1 varices	13 (18)	
	Grade 2 varices	1 (1)	
	PHG	21 (30)	

CI, confidence interval; BMI, body mass index; MELD, model of end-stage liver disease; HCC, hepatocellular carcinoma; PHG, portal hypertensive gastropathy. † No history of excessive alcohol consumption

Table 2. Biochemical changes with DAA-therapy.

	Baseline	Week 1	End of treatment	One-year
ALT (IU/L)	77 (66-87)	43 (36-49) *	24 (21-27) *	24 (21-28) *
Bilirubin (umol/L)	10 (9-11)	17.1 (14.7-19.5) *	11 (10-13) *	9 (7-10) *
Alkaline phosphatase (IU/L)	98 (88-107)	92 (83-101) *	84 (77-92) *	91 (82-100) *
Albumin (g/L)	35.4 (34.2-36.5)	35.3 (34.1-36.5)	35.7 (34.5-36.8)	37.5 (36.2-38.7) *
Creatinine (umol/L)	70 (65-74)	71 (66-76)	72 (67-76)	70 (65-74)
Leukocytes (x10^9/L)	6.0 (5.5-6.5)	6.7 (6.1-7.3) *	5.9 (5.4-6.4)	6.6 (6.0-7.1) *
Haemoglobin (mmol/L)	8.8 (8.5-9.0)	8.5 (8.2.8.8) *	7.4 (7.2-7.6) *	8.8 (8.5-9.1)
Platelets (x10^9/L)	137 (123-152)	148 (132-164) *	170 (151-188) *	161 (143-178) *
INR	1.2 (1-1-1.2)	1.2 (1.1-1.2)	1.2 (1.1-1.2)	1.1 (1.1-1.2)
Virus load (x10^3 IU/mL)	805 (398-1200)	0.3 (0.1-0.4) *	0*	0*
Alpha-foetoprotein	4.8 (3.7-5.8)	-	3.2 (2.5-3.9)*	2.7 (2.1-3.4)*

Data are reported as medians with (95% CI). *p<0.05 compared with the baseline values.

Figure legends

Figure 1. The dynamics of the macrophage activation markers, sCD163 and sMR at baseline, at designated weeks during treatment (1, 2, 4, 8), at the end of treatment (EOT) and after treatment (12-weeks and one-year post-treatment cessation). The dots represents medians with 95% CI based on a mixed model.

Figure 2. Liver stiffness in the patients with chronic hepatitis C at baseline, at the end of treatment (EOT), at 12-weeks post-treatment and one-year post-treatment, reported as relative changes from baseline. The patients are divided into the following groups: patients with advanced fibrosis (n=15), Child-Pugh A cirrhosis patients (n=49) and Child-Pugh B patients (n=7).

Figure 3. Galactose elimination capacity (GEC) reported in percentage of normal values in the patients with chronic hepatitis C at baseline, at 12-weeks post-treatment and one-year post-treatment. The dots represents means with 95% CI based on a mixed model.
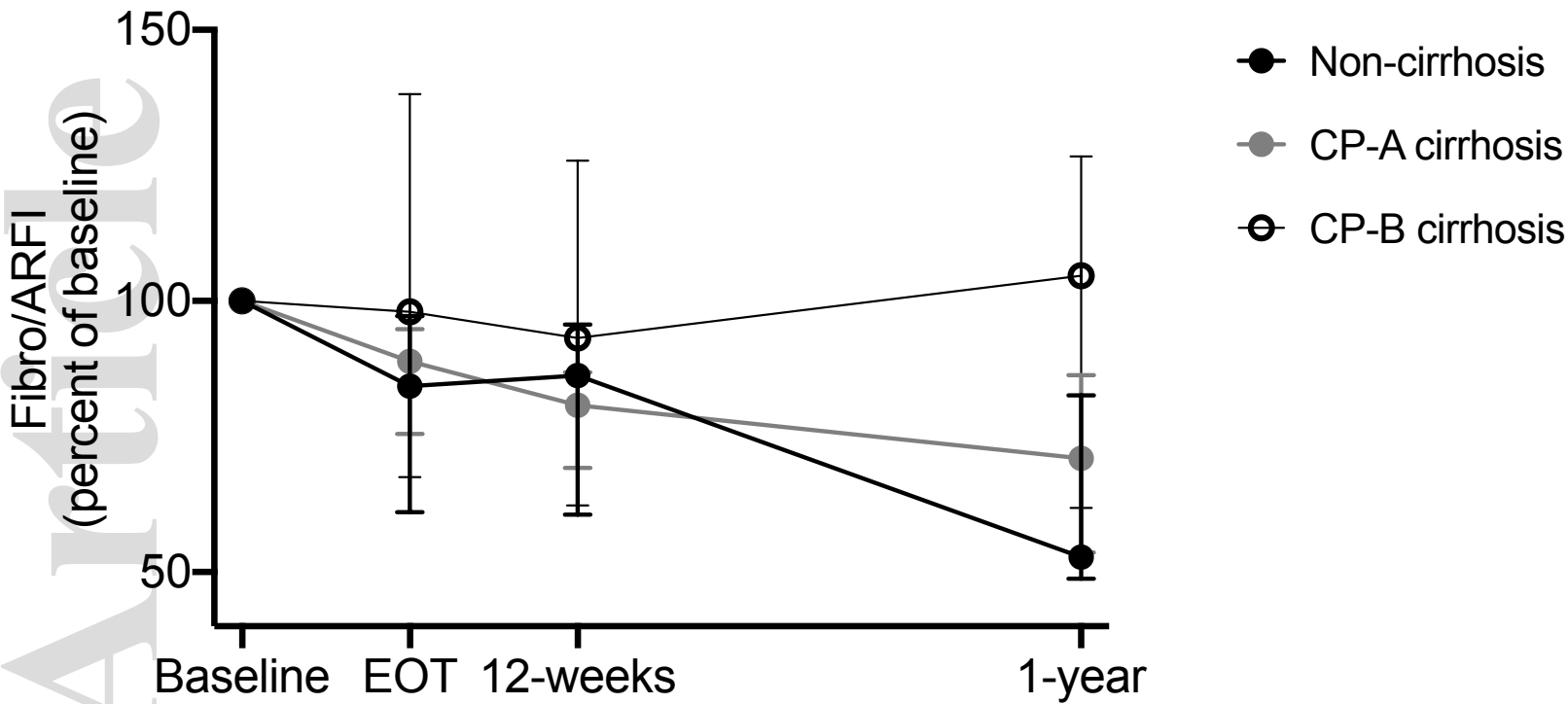
Figure 4. The continuous reaction time (CRT) index in the patients with chronic hepatitis C at baseline, and at 12-weeks and one-year after treatment cessation. The dots represents means with 95% CI based on a mixed model. The dashed line represents the lower limit of the normal interval.
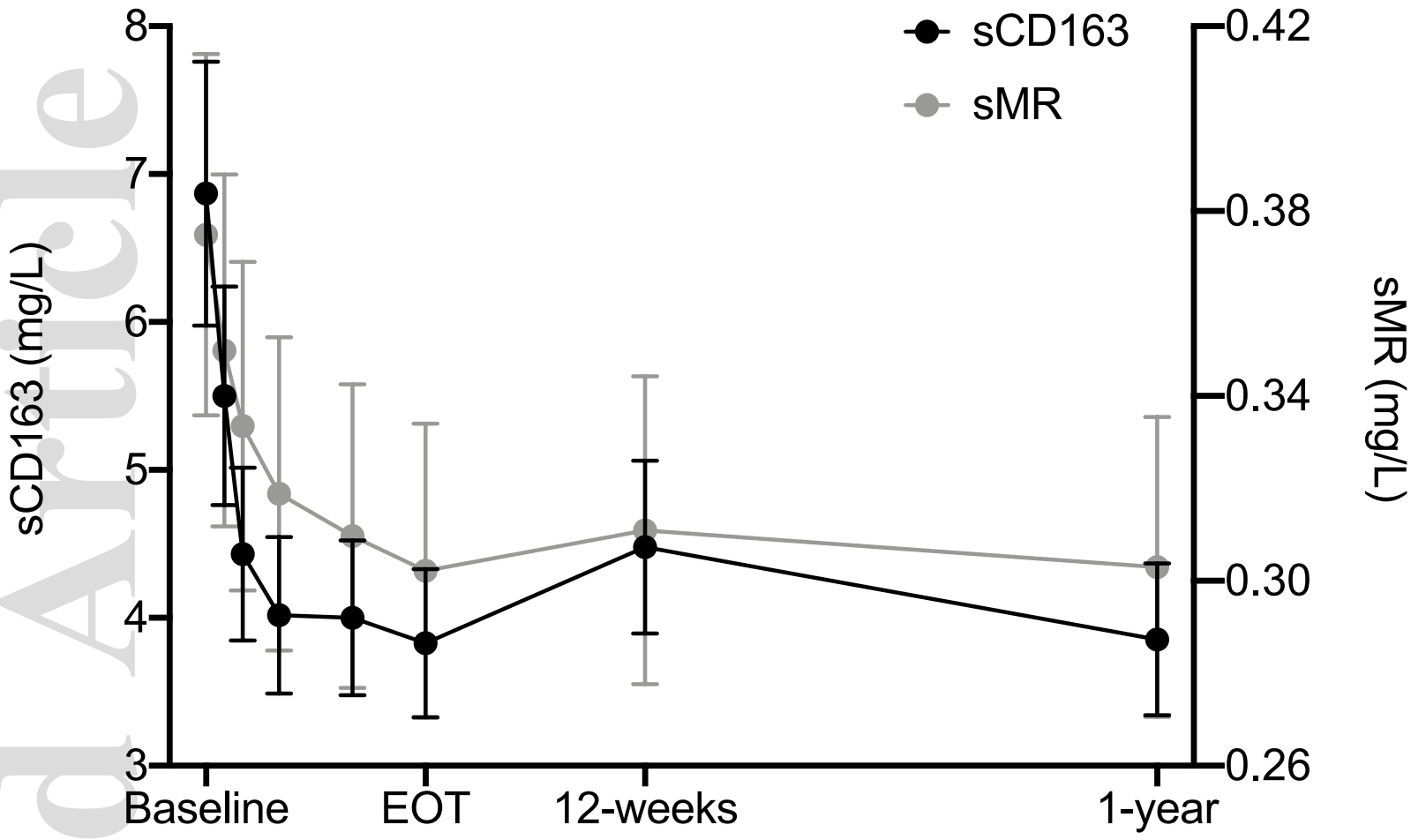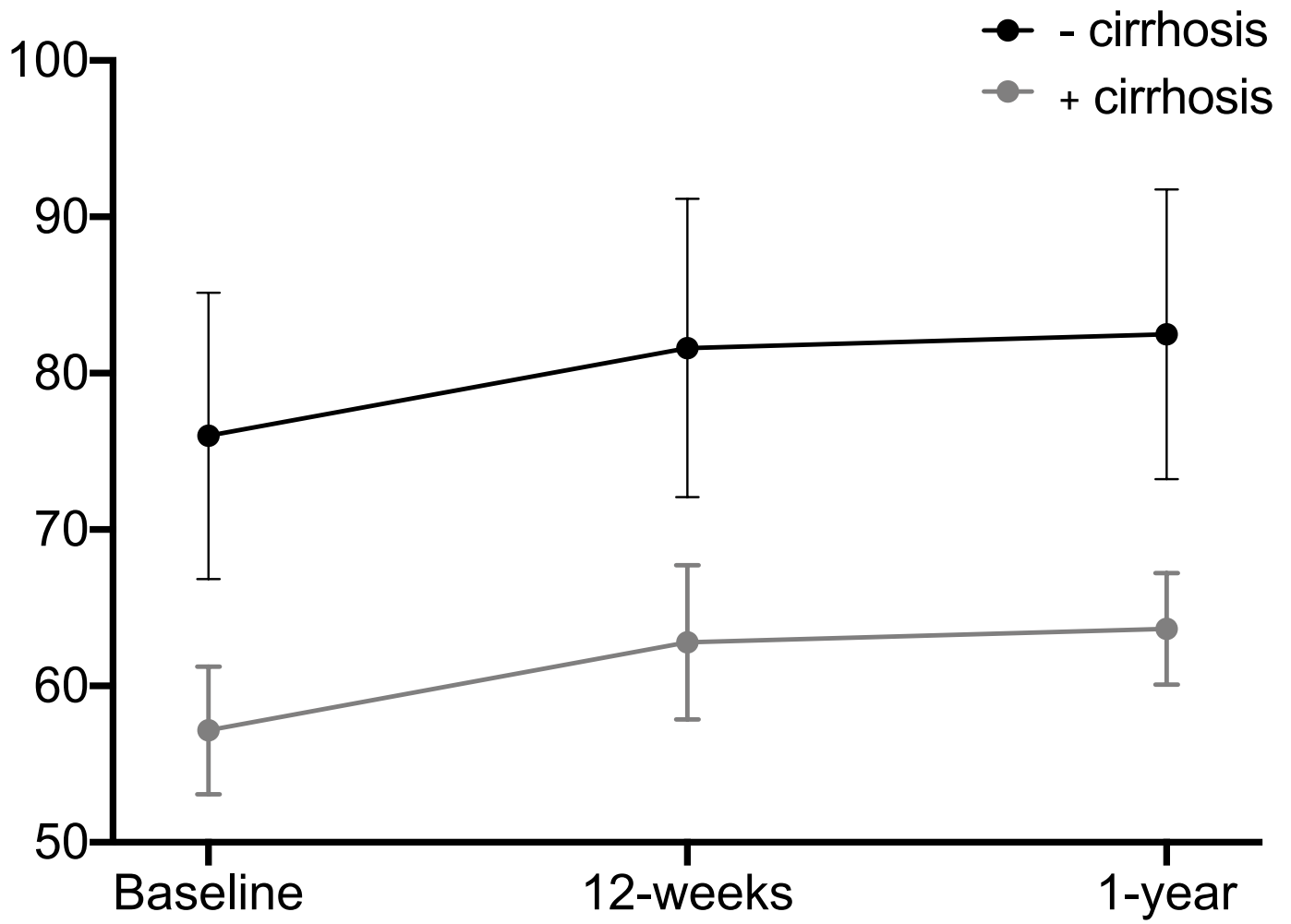
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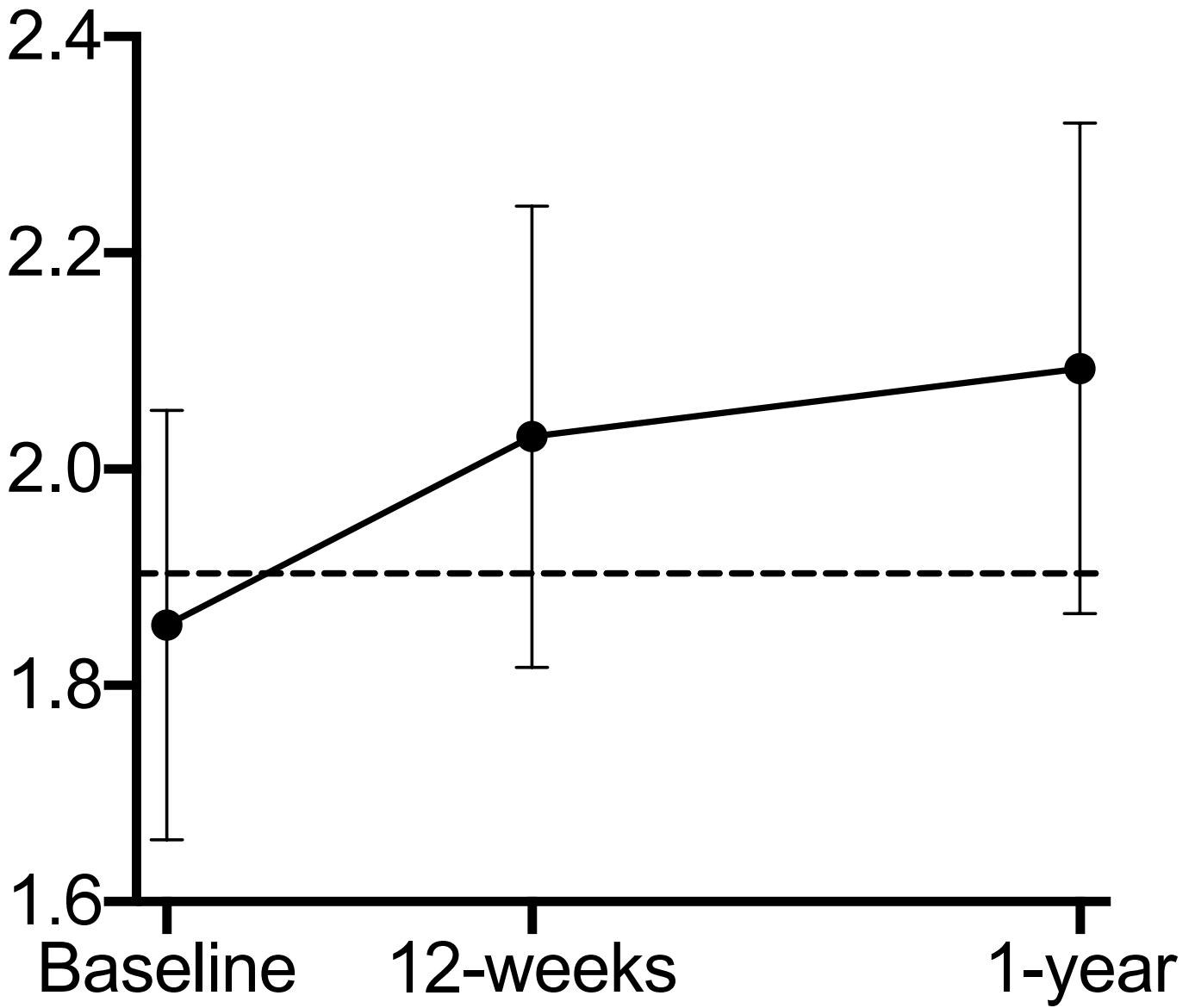
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