Effects of direct-acting antiviral therapy on the noninvasive markers of liver fibrosis and portal hemodynamics in liver transplant recipients

PhD thesis

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I. Introduction

Chronic hepatitis C (HCV) infection is one of the most common indications for liver transplantation in the Western countries. Reinfection of liver allografts is universal in patients with pre-transplantation viremia. While the diagnosis of recurrent HCV infection is defined by the presence of HCV RNA in serum and/or liver, the diagnosis of recurrent disease requires histological confirmation. The course of the disease is more aggressive in liver transplant recipients than in non-transplanted HCV-cirrhotic patients mainly due to the applied immunosuppressive therapy. HCV-related allograft cirrhosis develops in approximately 25% of recipients within 5 years after liver transplantation.

The introduction of direct-acting antiviral agents (DAA) has revolutionised HCV therapy, as sustained virologic response rates (SVR) over 90% are achievable both in non-transplant patients and in liver transplant recipients. The degree of liver fibrosis and portal hypertension highly influence graft and patient survival, therefore they should be monitored after antiviral treatment. The impact of HCV eradication on the degree of liver fibrosis has been widely investigated in recent years. However, there is not much known about portal hemodynamic alterations after antiviral treatment. In the assessment of fibrosis, liver biopsy is the reference standard, while portal pressure can be measured via catheterisation. Both liver biopsy and transcatheter portal pressure measurement are invasive procedures with potential life-threatening complications. The most prevalent noninvasive methods to assess liver fibrosis are elastography techniques and peripheral biomarkers. Transcatheter portal pressure measurement can be substituted by liver elastography, splenic elastography, splenic volume assessment, Doppler ultrasonography and peripheral biomarkers. Besides liver fibrosis and portal hemodynamics, hepatocellular carcinoma development also highly influences patients' life expectancy. The impact of DAA therapy on HCC risk is a controversial topic even nowadays.

II. Aim of the study

In our study we aimed to answer the following questions:

1. How effective is DAA therapy in the treatment of HCV recurrence?

2. Does the antiviral therapy influence noninvasive markers of liver fibrosis and steatosis?

3. Does the antiviral therapy influence noninvasive markers of portal hypertension?

4. Does the antiviral therapy impact the development of de novo HCC or recurrent HCC?

III. Methods

III.1 Patients and protocol

Thirty-eight liver transplant recipients with HCV recurrence of the Department of Transplantation and Surgery, Semmelweis University have been enrolled in this prospective study. All patients underwent 24-week ombitasvir/paritaprevir/ritonavir + dasabuvir +/- ribavirin combination therapy. Ultrasonography, non-contrast Computed Tomography, laboratory and 2D shear wave elastography examinations were performed at three time-points: the day before the beginning of the antiviral treatment (Baseline), at the end of the traetment (EOT) and 24 weeks after the end of the treatment (SVR 24). Virological response was assessed by a quantitative HCV RNA assay (COBAS Taqman 48, Roche). Characteristics of study participants are summarized in Table 1. The study protocol was approved by the Hungarian Ethical Committee.

1. Table: Patient data

Age	60.8±4.5 years
Sex	male/female: 24/14
Time elapsed between transplantation and Baseline	4.9±4.5 years
Indication of transplantation	HCV cirrhosis: 28 patients HCV cirrhosis and HCC: 9 patients HCV and HBV coinfection: 1 patient
Interferon treatment history	Treatment naive: 19 patients Non-responder: 16 patients Relapser: 3 patients
HCV genotype	1a: 2 patients 1b: 34 patients 1, subtype can not be determined: 2 patients
Child-Pugh score	Child A: 35 patients Child B: 3 patients

III.2 2D shear wave elastography (2D SWE)

2D-SWE examinations were performed with an Aplio 500 ultrasonography machine (Toshiba Medical Systems, Tochigi, Japan) equipped with an SWE module. After being fasted overnight, all patients were examined in supine position with a convex ultrasound probe via a right intercostal approach. A sample box was positioned in the right lobe of the liver. Liver stiffness values were calculated in kPa automatically by the software (Figure 1). Median value of 10 consecutive measurements for each patient was used for statistical analysis.

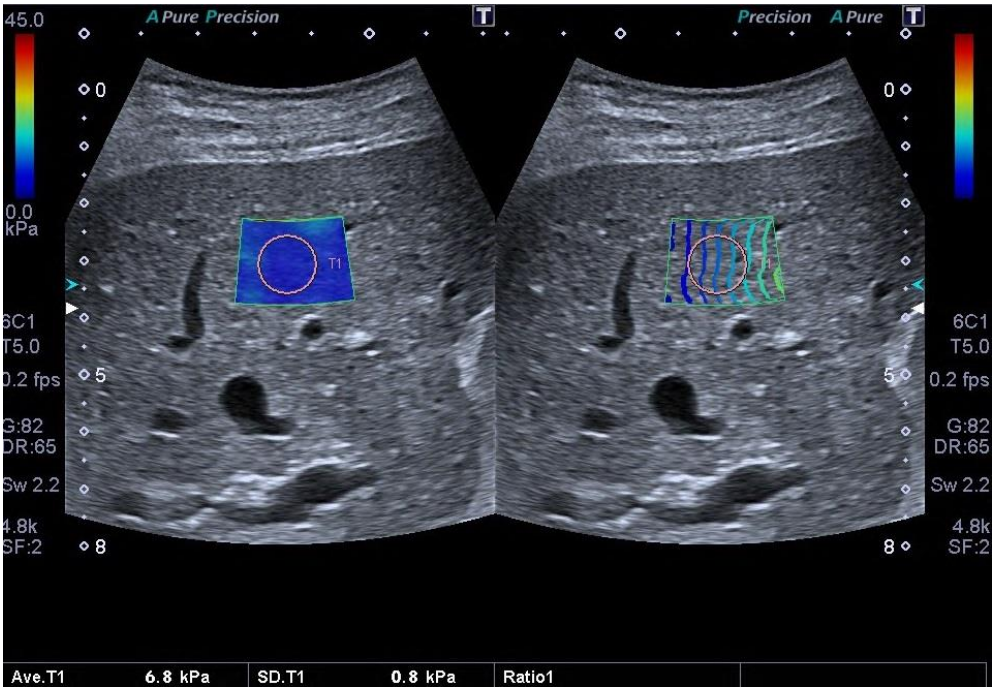

Figure 1: 2D SWE examination of the liver. A trapezoid sample box was placed at a location more than 1 cm below the liver capsule, away from large vessels. A circular, 1-cm-large region of interest was placed within the speed map and concordant propagation map simultaneously to obtain the mean speed of the shear-wave (2nd Department of Internal Medicine, Semmelweis University)

III.3 CT volumetry

Non-contrast abdominal CT scans were performed by Philips Brilliance 16 CT Scanner (Philips Medical Systems, the Netherlands). Splenic volume (cm^3) was determined by CT volumetry (Figure 2) using the Extended Brilliance Workspace environment (EBW version 2, Philips Healthcare, Cleveland, USA). The spleen was contured on each axial CT slices manually, where slice thickness was 5 mm. Splenic volume was then calculated automatically by the software.

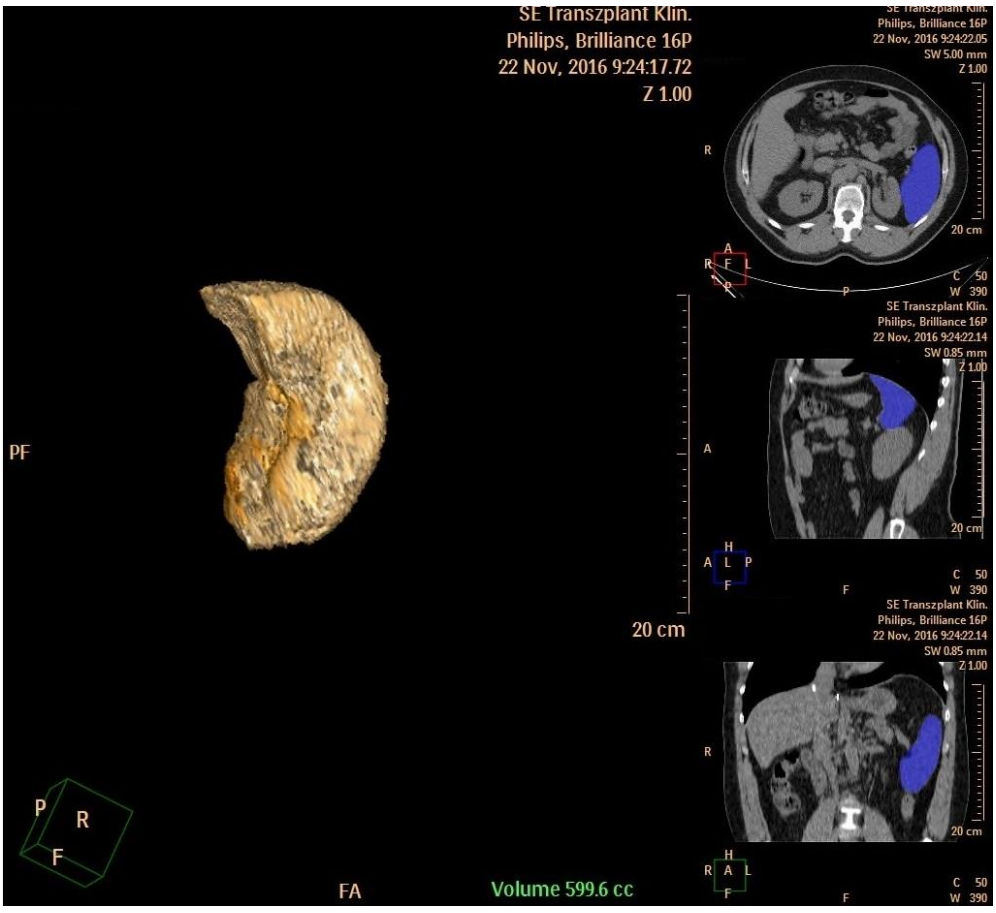

Figure 2: Calculation of splenic volume using CT volumetry. (Department of Transplantation and Surgery, Semmelweis University)

III.4 CT attenuation measurements

CT attenuation measurements were performed on noncontrast CT images. Minimum 2-cm-large ROIs were placed in both liver lobes and the spleen (Figure 3). Liver CT attenuation was defined as the average attenuation of both lobes. Liver-to-spleen (L/S) attenuation ratio was calculated.

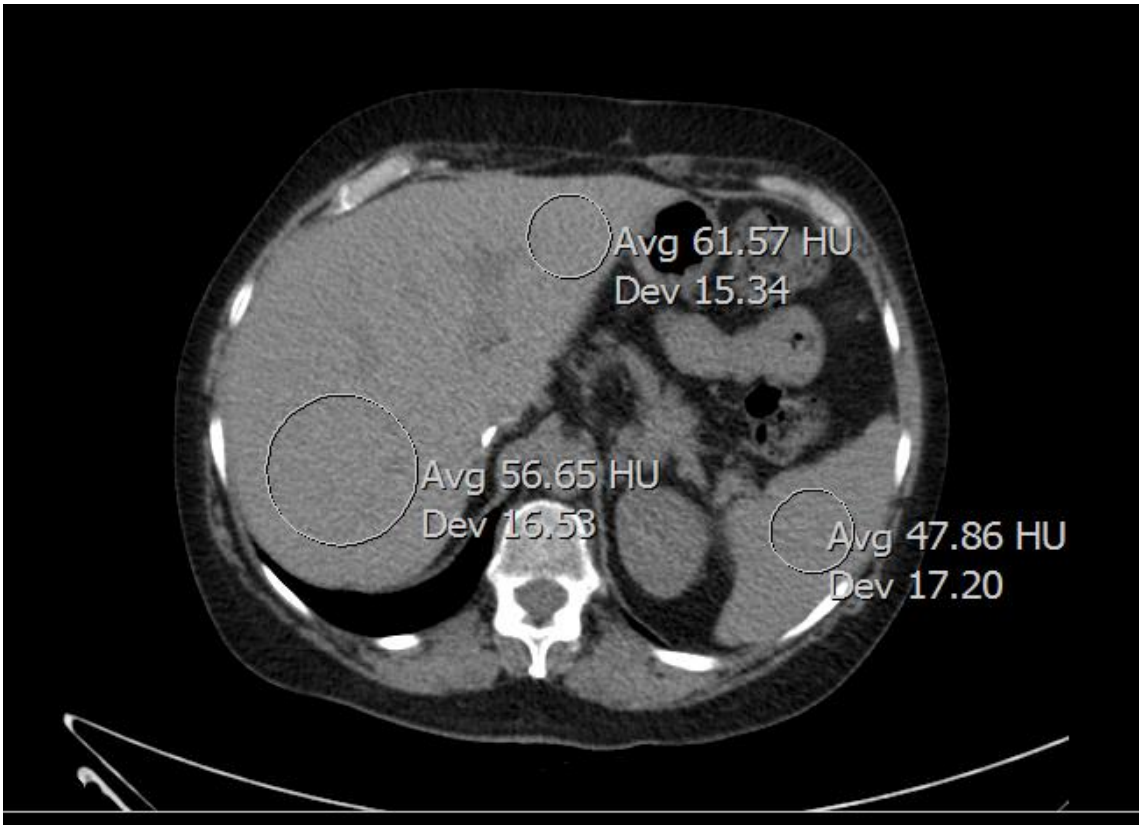

Figure 3: CT attenuation measurements (Department of Transplantation and Surgery, Semmelweis University)

III.5 Abdominal ultrasound scan

The liver and the bile ducts were studied using a Toshiba Xario 50 ultrasound system (Toshiba Medical Systems, Japan). After an overnight fasting all patients were examined in the supine and in the left decubitus position with a convex ultrasound probe (1-5 MHz). The liver parenchyma was scanned in two planes from the subcostal approach and it was also examined from the intercostal approach. The aim of the study was to detect focal liver lesions. After scanning the entire liver parenchyma we examined the intra- and extrahepatic bile ducts in order to exclude cholestasis. The following regions were evaluated to assess the presence of free abdominal fluid: around the liver, around the spleen, in both paracolic spaces and in the pelvis. The quantity of ascities was assessed subjectively.

III.6 Doppler ultrasound measurements

B-mode and Color Doppler ultrasound examinations were performed using a Toshiba Xario 50 ultrasonography machine (Toshiba Medical Systems, Japan). After an overnight fasting all patients were examined in supine position with a convex ultrasound probe (1-5 MHz). Liver vessels were examined via a right, while splenic vessels via a left intercostal oblique approach. Portal vein diameter (PVD) and flow velocity were measured 1 cm proximal to its bifurcation. Portal blood flow velocity (PBV) was evaluated as the time-averaged maximum velocity multiplied by the coefficient 0.57, assuming the portal velocity profile to be parabolic. Hepatic and splenic Doppler waveforms were obtained in the hepatic and splenic hilum, respectively. Color Doppler allowed the identification of the main arterial branches. The sample volume of the Doppler system was placed inside these vessels, near the hilum, and the blood flow velocity waveform was recorded. Peak systolic, end diastolic and temporal mean flow velocity were then determined for both the hepatic and splenic artery.

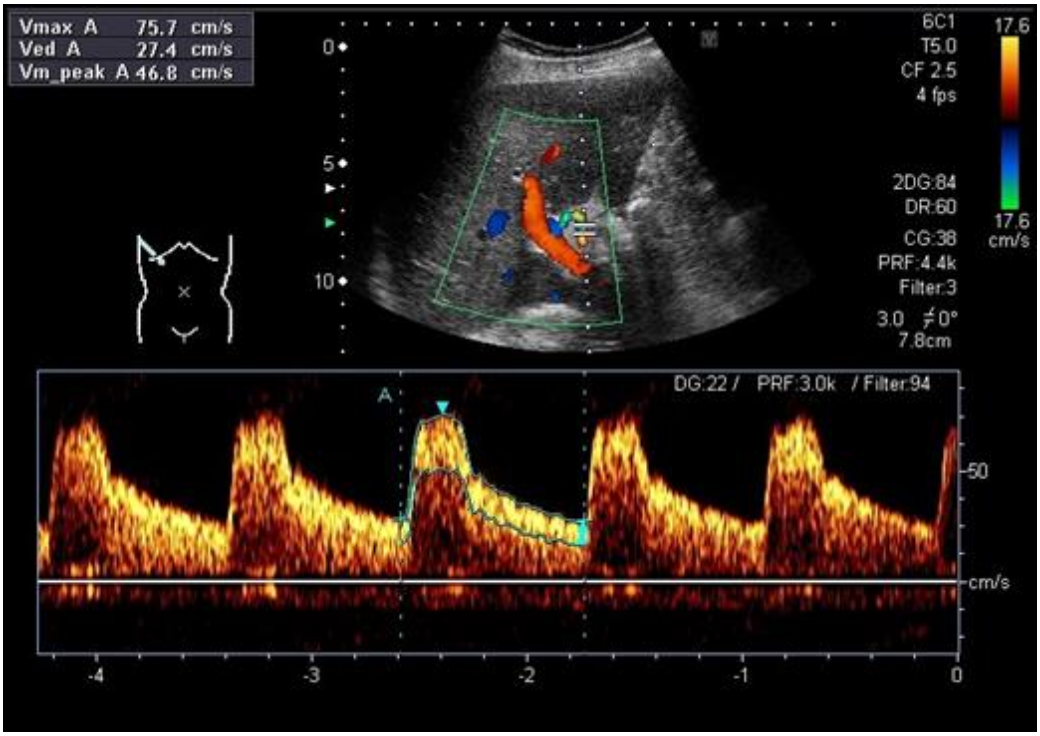

Figure 4: Hepatic artery spectral waveform. (Vmax A: peak systolic velocity; Ved A: end diastolic velocity; Vm_peak A: mean velocity; Department of Transplantation and Surgery, Semmelweis University)

The resistive index (RI), the pulsatility index (PI) and the congestion index (CI) were calculated in each patient using the following equations:

RI = (peak systolic velocity - end diastolic velocity) / peak systolic velocity

PI = (peak systolic velocity − end diastolic velocity) / mean velocity

CI = portal vein cross-sectional area / mean portal vein velocity

III.7 Serum biomarkers

Serum biomarkers of liver fibrosis were calculated using the following equations:

APRI (AST to platelet ratio index): GOT [U/L]/upper limit of GOT [U/L]/platelet count [10^9/L] x 100;

FIB-4 (Fibrosis-4 score): (age [years] x GOT [U/L])/(platelet count [10^9/L] x GPT [U/L]$^{1/2}$).

A serum biomarker of portal hypertension was calculated as follows:

Risk score: 14.2 - 7.1 x log(10) (platelet count [10^9/L]) + 4.2 x log(10) (bilirubin [mg/dL])

III.8 Statistical analysis

Data were analysed using GraphPad Prism 6 (GraphPad Inc, La Jolla, USA) statistical software. One-way ANOVA was used to compare the studied parameters in the different timepoints. Correlation between liver elasticity and serum biomarkers of liver fibrosis was tested with Spearman's rank correlation test. Data were expressed as mean±1SD, $p<0.05$ was considered significant.

IV. Results

IV.1 Treatment response

DAA therapy was well tolerated with no major side-effect registered. The treatment did not have to be stopped prematurely in any of the cases. All patients became HCV RNA negative by the end of the DAA treatment, and had a sustained virologic response 24 weeks after the end of the treatment.

IV.2 2D shear wave elastography (2D SWE)

Median liver elasticity decreased significantly at SVR 24 compared to Baseline (10.68±11.46 vs. 7.17±3.45, $p<0.05$) (Figure 5).

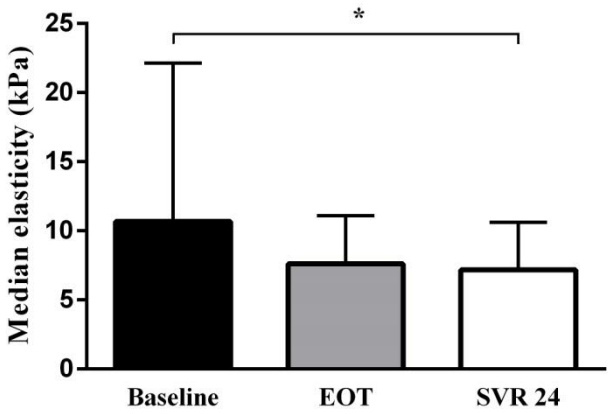

Figure 5: Median liver elasticity at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. Median liver elasticity decreased significantly at SVR 24 compared to Baseline (*: $p<0.05$).

According to cut-off values in the literature 22/37 patients at Baseline, 16/37 patients at EOT and 10/37 patients at SVR 24 had significant fibrosis (F2-4) based on liver elasticity values. Liver cirrhosis (F4) was present in 9/37 cases at Baseline and in 3/37 cases each at EOT and SVR 24 (Figure 6).

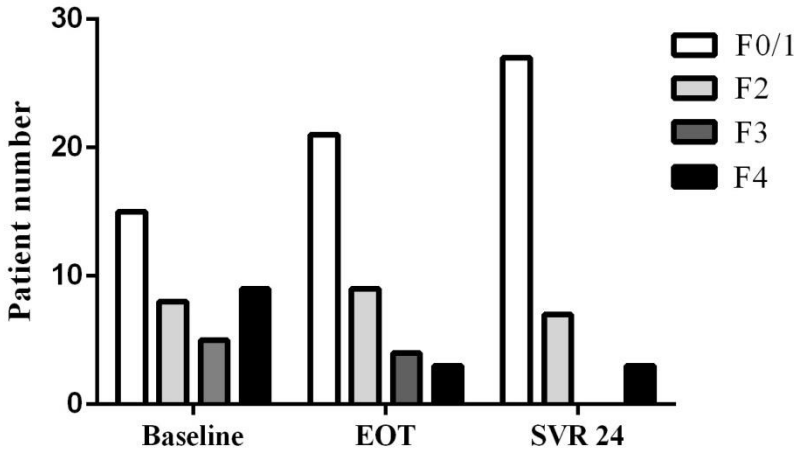

Figure 6: Patients' Metavir score based on liver elasticity values at Baseline, EOT and SVR 24.

IV.3 CT volumetry

One of our patients had splenectomy due to childhood trauma, in this case splenic volume could not be determined. Splenic volume measured by CT volumetry decreased significantly at SVR 24 compared to Baseline and EOT (466.3±189.0 cm^3 vs. 413.5±166.9 cm^3, $p<0.0001$ and 453.3±183.9 cm^3 vs. 413.5±166.9 cm^3, $p<0.0001$, respectively) (Figure 7). According to literature the normal range of splenic volume is between 107 and 314 cm^3. At Baseline 80,6% (29/36), at EOT 77,8% (28/36), at SVR 24 69,4% (25/36) of our patients had a splenic volume above the upper limit.

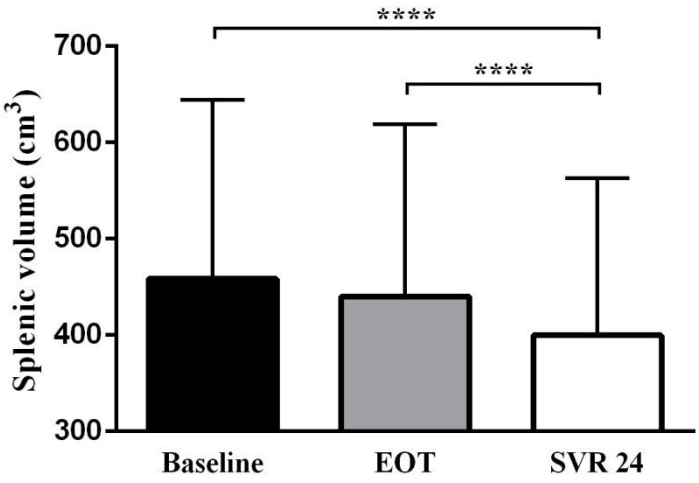

Figure 7: Splenic volume at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. Splenic volume decreased significantly at SVR 24 compared to Baseline and EOT (****: $p<0.0001$).

IV.4 CT attenuation measurements

Splenic CT attenuation and L/S ratio could not be determined in the patient who had slenectomy due to childhood trauma. L/S ratio increased significantly at EOT and SVR 24 compared to Baseline (1.18±0.14 vs. 1.30±0.13, $p<0.0001$ and 1.18±0.14 vs. 1.27±0.13, $p<0.01$, respectively) (Figure 8). According to literature an L/S ratio above 1.0 indicates liver steatosis. At Baseline 8,3% (3/36), while at EOT and SVR 24 none of our patients had an L/S ratio above 1.0.

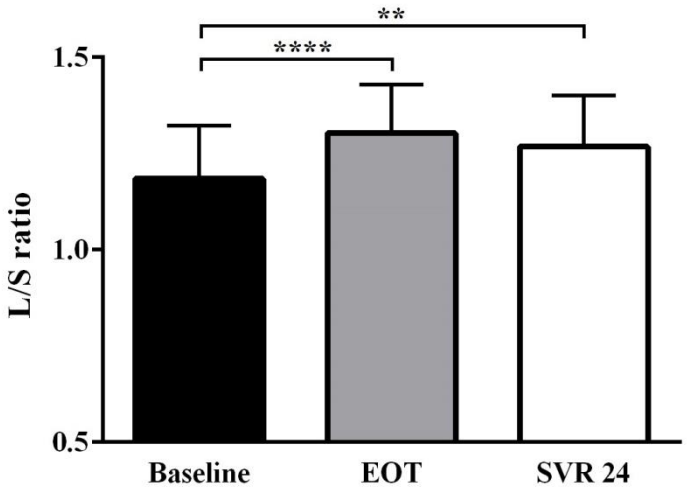

Figure 8: L/S ratio at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. L/S ratio increased significantly at EOT and at SVR 24 compared to Baseline (**:$p<0.01$; ****: $p<0.0001$).

IV.5 Abdominal ultrasound scan

A liver lesion suspicious for malignancy was detected in none of the cases. One of our patients had moderate bile duct dilatation at EOT, therefore he was excluded from the study. Free abdominal fluid was present in 4 cases at Baseline and in 1 case each at EOT and SVR 24.

IV.6 Doppler ultrasound measurements

The studied ultrasound and Doppler ultrasound parameters are summarised in Table 2.

Table 2: The studied circulatory parameters at Baseline, EOT and SVR 24 (mean±1SD).

	Baseline	EOT	SVR 24
Mean portal blood flow velocity (cm/s)	18.76±7.69	18.52±8.28	15.33±6.29
Portal vein diameter (cm)	1.26±0.39	1.24±0.30	1.27±0.33
Hepatic artery RI	0.72±0.09	0.75±0.07	0.73±0.08
Hepatic artery PI	1.36±0.48	1.58±0.44	1.48±0.43
Splenic artery RI	0.66±0.06	0.66±0.07	0.69±0.06
Splenic artery PI	1.14±0.23	1.19±0.33	1.23±0.27
Congestion index (cm*s)	0.07±0.03	0.07±0.03	0.11±0.10

Mean portal blood flow velocity decreased significantly at SVR 24 compared to Baseline and EOT (18.76±7.69 cm/s vs. 15.33±6.29 cm/s, $p<0.001$ and 18.52±8.28 cm/s vs. 15.33±6.29 cm/s, $p<0.05$, respectively). Portal vein diameter and congestion index did not change significantly. Among the studied arterial Doppler indices only the hepatic artery pulsatility index changed significantly: at EOT it was permanently elevated compared to Baseline (1.36±0.48 vs. 1.58±0.44, p<0,05).

IV.7 Serum biomarkers

IV.7.1 Serum biomarkers of liver fibrosis

APRI score decreased significantly at EOT and SVR 24 compared to Baseline (1.28±1.72 vs. 0.29±0.18, p<0.01 and 1.28±1.72 vs. 0.32±0.21, p<0.01, respectively) (Figure 9). An APRI score higher than 0.7 indicates significant fibrosis, while an APRI score higher than 1.0 indicates severe fibrosis or cirrhosis. At Baseline 21.6% (8/37) of our patients had an APRI score between 0.7-1.0 and 35.1% (13/37) of our patients had an APRI score above 1.0. At EOT 5.4% (2/37), at SVR 24 8.1% (3/37) of our patients had an APRI score between 0.7-1.0. None of our patients had an APRI score above 1.0 at EOT or SVR 24.

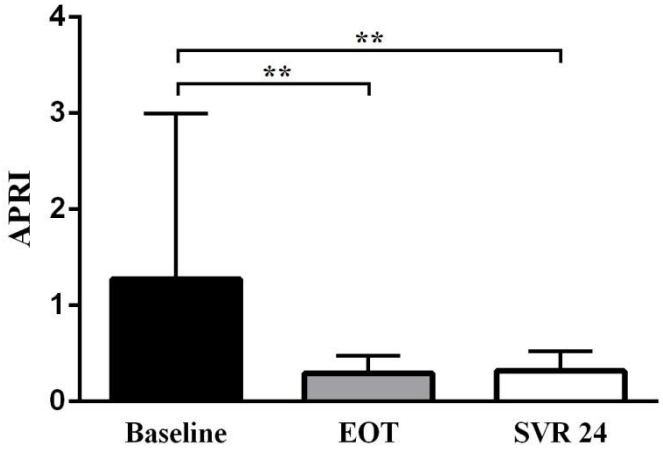

Figure 9: APRI score at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. APRI score decreased significantly at EOT and SVR 24 compared to Baseline (**: p<0.01).

FIB-4 index decreased significantly at EOT and SVR 24 compared to Baseline (2.79±2.01 vs. 1.45±0.69, p<0.0001 and 2.79±2.01 vs. 1.47±0.71, p<0.001, respectively) (Figure 10). According to literature a FIB-4 index below 1.45 excludes severe fibrosis, while a FIB-4 index above 3.25 indicates severe fibrosis or cirrhosis (F3-4). FIB-4 index indicated severe fibrosis or cirrhosis in 29.7% (11/37) of our patients at Baseline and in 2.7% (1/37) of our patients at EOT and SVR 24.

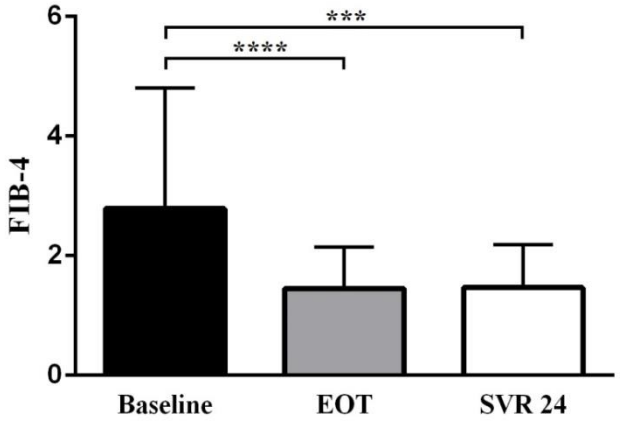

Figure 10: FIB-4 index at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. FIB-4 index decreased significantly at EOT and SVR 24 compared to Baseline (***: p<0,001; ****: p<0,0001).

IV.7.2 Serum biomarker of portal hypertension

Risk score decreased significantly at EOT and SVR 24 compared to Baseline (-1.73±1.49 vs. -2.13±1.51, p<0.05 and -1.73±1.49 vs. -2.45±1.48, p<0.01, respectively) (Figure 11). The optimal cut-off value of Risk score for significant portal hypertension is -1. At Baseline 37.8% (14/37), at EOT 18.9% (7/37), at SVR 24 13.5% (5/37) of our patients had a Risk score above -1.

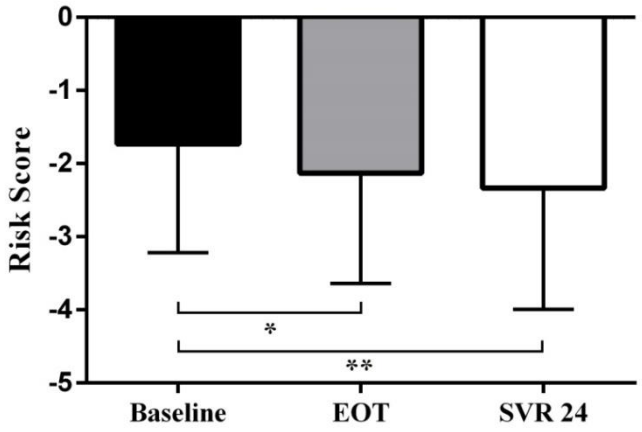

Figure 11: Risk score at Baseline, EOT and SVR 24. Data were expressed as mean±1SD. Risk score decreased significantly at EOT and SVR 24 compared to Baseline (*: p<0,05; **: p<0,01).

V. Conclusions

Based on the results obtained, the following conclusions can be made:

1. The applied antiviral therapy was highly effective (SVR: 100%) and well-tolerated in the studied patient cohort.

2. After DAA therapy the studied noninvasive markers of liver fibrosis and steatosis showed a significant improvement. As far as we know, this is the first study that described an improvement in liver stiffness values measured by 2D shear wave elastography in liver transplant recipients treated for HCV recurrence. Moreover, this is the first study that described beneficial changes of hepatic CT attenuation in the same patient cohort. Our results indicate that the degree of liver fibrosis and steatosis decreases after successful antiviral therapy in liver transplant recipients.

3. Among the noninvasive markers of portal hypertension splenic volume and Risk score showed a significant improvement, while the prevalance of ascites decreased. To the best of our knowledge no other authors have found that splenic volume decreases after successful DAA therapy. Our results suggest that HCV ereadication can lead to beneficial changes in portal hemodynamics, presumably as a result of fibrosis regression.

4. After DAA therapy no suspicious lesion for HCC was detected in our patients.

VI. Summary

In liver transplant recipients HCV recurrence is a major cause of graft loss and mortality. The introduction of direct-acting antiviral agents (DAA) has revolutionised HCV therapy, as high sustained virologic response rates are achievable even in the liver transplant setting. Our aim was to study the effects of DAA therapy on non-invasive markers of liver fibrosis, steatosis, portal hypertension in liver transplant recipients with HCV recurrence. We also evaluated the impact of antiviral therapy on hepatocellular carcinoma (HCC) risk.

Thirty-seven liver transplant recipients with HCV recurrence have been enrolled in this prospective study. All patients underwent 24-week ombitasvir/paritaprevir/ritonavir + dasabuvir +/- ribavirin combination therapy. Ultrasonography, computed tomography, shear-wave elastography and laboratory examinations were performed at the beginning of the antiviral treatment (Baseline), at the end of the treatment (EOT) and 24 weeks after the end of the treatment (SVR 24). Virological response was assessed by a quantitative HCV ribonucleic acid assay.

The applied antiviral treatment was highly effective (SVR: 100%) and well-tolerated. Liver stiffness values improved significantly after the antiviral treatment, 42% of the patients were transferred to a more favourable Metavir stage based on liver elasticity values. Serum biomarkers of liver fibrosis, patients' splenic volume and the studied serum biomarker of portal hypertension (Risk score) also showed a significant improvement. The prevalence of ascites decreased, while L/S ratio (liver-to-spleen CT attenuation ratio) increased significantly. Suspicious lesion for HCC occured in none of the cases.

The improvement of liver stiffness values and serum biomarkers of liver fibrosis suggests that the transplanted liver is capable of undergoing a relatively fast regeneration after virus eradication. The observed alteration in CT attenuation values implies that liver fat content decreased after DAA therapy. The reduction of splenic volume and Risk score indicate beneficial changes of portal hemodynamics. The regression of liver fibrosis and the improvement of portal hemodynamics might have an important role in long term graft and patient survival.

VII. Publication list

VII.1 List of publications related to the dissertation

1: **Korda D**, Lenard ZM, Gerlei Z, Jakab Z, Haboub-Sandil A, Wagner L, Varga M, Cseprekal O, Marton A, Horvathy D, Takacs S, Doros A, Mathe Z. Shear-wave elastography for the assessment of liver fibrosis in liver transplant recipients treated for hepatitis C virus recurrence. Eur J Gastroenterol Hepatol. 2018 Jan;30(1):27-32.

2: **Korda D**, Deák PÁ, Kiss G, Gerlei Z, Kóbori L, Görög D, Fehérvári I, Piros L, Máthé Z, Doros A. Management of Portal Hypertension After Liver Transplantation. Transplant Proc. 2017 Sep;49(7):1530-1534.

VII.2 List of publications not related to the dissertation:

3: **Korda D**, Deák PÁ, Kozma V, Kiss G, Doros A. Role of Contrast-Enhanced Ultrasound in the Follow-up of Kidney Transplant Patients. Transplant Proc. 2016 Sep;48(7):2544-2547.

4: **Korda D**, Doros A, Piros L, Gerlei Z, Haboub-Sandil A, Mándli T, Fazakas J, Deák ÁP, Máthé Z. Liver Transplant for Metastatic Neuroendocrine Tumors: A Single-Center Experience in Hungary. Transplant Proc. 2019 May;51(4):1251-1253.

5: Kiss G, **Korda D**, Szabó G, Juhász R, Wagner L, Máthé Z, Doros A, Végső G. Oncological Screening of Kidney Transplant Patients: The Role of Ultrasound Examination. Transplant Proc. 2019 May;51(4):1231-1233.

6: Farkas ÁZ, Török S, Kovács JB, Piros L, Végső G, Kiss G, **Korda D**, Bibok A, Hartmann E, Deák ÁP, Doros A. Diagnosis and Management of a De Novo Urothelial Carcinoma in a Kidney Allograft: A Case Report. Transplant Proc. 2019 May;51(4):1281-1285.

7: Deák PÁ, **Korda DÁ**, Doros A. [Thermoablation therapy in the treatment of benign lesions]. Orv Hetil. 2016 Dec;157(51):2040-2047. Hungarian.