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Original article

# Sustained virological response by direct antiviral agents in HCV leads to an early and significant improvement of liver fibrosis

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## Abstract

**Background: Direct Antiviral Agents (DAA) demonstrated high efficacy among HCV-infected patients in registered trials. Nevertheless, the impact of these therapies on liver stiffness measurement(LSM) and liver functionality in "real-life" is not well-known. Aim of the present study was to evaluate the SVR impact on LSM and clinical parameters of DAA-therapy on a real-life population of HCV patients with F3/F4 fibrosis.**

**Methods: 749 HCV genotype 1-4 patients with F3/F4 hepatitis undergoing antiviral therapy, were consecutively enrolled in four centers of Hepatology of Italy. Clinical, biochemical and imaging data were collected at the baseline(T0), at the End of Therapy(EoT) and after 12 weeks(SVR12).**

**Results: Out of 749 patients, 69.7% was F4 and 30.3% was F3. SVR12 was reached in 97,5%. LSM significantly decreased from T0 to EoT($p<0.001$) whereas it did not from EoT to SVR12(p:ns). Moreover, in F4 no significant differences were found in Child and MELD between T0, EoT and SVR12(p=ns). At the univariate analysis of clinical and liver parameters, baseline high glucose($p<0.005$), type 2 diabetes($p<0.001$), low ALT($p<0.001$), low PLTs($p<0.005$), and the presence of esophageal varices (EV)($p<0.001$) were found to be associated with a lack of a significant EoT LSM improvement. At a multiple regression, ALT($p<0.05$), Diabetes($p<0.005$) and EV($p<0.05$), were inversely associated with significant LSM reduction.**

**Conclusions: Virological response to DAA is associated with fibrosis regression and recovery of liver functionality and this can be detected as early as EoT. HCV**

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**eradication is associated with a rapid and significant clinical improvement that lasts overtime and seems to be negatively influenced by diabetes and EV.**

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Running head: DAA in HCV: viral eradication leads to an early improvement of fibrosis

## Introduction

Thirty years after the discovery of hepatitis C virus (HCV), the global importance of HCV is well established and chronic HCV infection, affecting all countries, is one of the main cause of liver cirrhosis, hepatocellular carcinoma (HCC) and, consequently, one of most common indications for liver transplantation [1]. Recently, interferon-free treatment regimens with 2nd generation direct-antiviral agents (DAAs) for treatment of chronic HCV infection demonstrated high efficacy in terms of viral eradication [2]. These novel therapies, targeting HCV NS3/4A protease (Simeprevir, Paritaprevir), NS5B RNA-dependent RNA polymerase (Sofosbuvir, Dasabuvir) and NS5A serine protease (Ledipasvir, Daclatasvir, Ombitasvir), have minimal side effects and allow better chances of treatment to patients with contraindications and/or low sustained virological response (SVR) rates to interferon $\alpha$-based regimens, such as “decompensated” cirrhotic or transplanted patients [3]. The achievement of viral eradication is very important for the infected patient, particularly for those suffering from the most advanced diseases. Indeed, sustained virological responding patients experience a significantly lower rate of liver decompensation and complications, hepatocellular carcinoma (HCC) and liver related deaths [4–6]. This evidence comes mainly from reports on interferon $\alpha$-based regimens, in which the possibility of a regression of the cirrhosis has been reported in 49% of subjects of a large pre- and post-treatment biopsy-proven study [7]. Moreover, a statistically significant reduction of portal hypertension has also been documented in SVR peg-IFN treated patients [8]. Recently, the data from studies on treatment with DAAs regimens in “advanced” cirrhotic patients have shown improvements of Child-Pugh-Turcotte (CPT) and model of end-stage-liver disease (MELD) scores in most of cases who obtained SVR, however a small number of those patients showed a worsening of these scores, despite a successful treatment [9,10]. Despite the extraordinary SVR prevalence, the clinical impact of the novel DAAs therapies in “real-life” in terms of liver decompensation, fibrosis regression and portal hypertension, particularly in patients with advanced fibrosis (F3-F4), is not yet well-known.

The gold standard for the staging of liver fibrosis is represented by liver biopsy. Its execution, however, is limited by invasiveness and a questionable inconstant accuracy due to inadequate specimens size, sampling variations and observer variability [11]. For this reason, non invasive techniques have been proposed for its assessment. Fibrosis-4 Score (FIB-4) and aspartate aminotransferase-platelet ratio index (APRI) scores are easily obtained on the basis of laboratory values and have been demonstrated to have a good sensitivity and specificity with the indisputable advantages in terms of time and costs in chronic HCV infection [12,13]. In addition, liver transient elastography (TE) was introduced in 2003 and has quickly become the main reference technique for the estimation of liver fibrosis, replacing the biopsy in most of cases [14–18]. TE is a fast and repeatable technique based on the quantization of liver stiffness. For this reason, TE has become a widely-used surrogate marker of liver fibrosis that is performed prior to the antiviral treatment initiation [19]. Nevertheless, TE results are influenced not only by liver fibrosis but also by necro-inflammation and portal hypertension [20–23]. Therefore, to perform TE prior to starting the treatment and at

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the follow-up may be useful to evaluate the clinical impact of SVR in DAA treated HCV patients. In this way, the aim of the present study is precisely to evaluate the impact of antiviral treatment on indirect markers of fibrosis and laboratory parameters of liver functionality after the achievement of HCV infection viral eradication in F3 and F4 patients treated with IFN-Free DAA.

# Patients and Methods

## Study design

Seven hundred and forty-nine patients with compensated cirrhosis (CHILD A/B) or advanced fibrosis (LSM≥10 kPascal) undergoing interferon-free antiviral therapy with direct antiviral agents for HCV, were consecutively enrolled from April 2015 to September 2016 in the present prospective, non interventional, multicenter observational study, conducted in four tertiary centers for the diagnosis and cure of chronic Hepatitis C in Southern Italy.

## Ethics statements

Every patient was included in the present study after obtaining a written informed consent. The present study was approved by our local ethics committee (Ethics committee of Campania Sud) and conducted in conformity with the ethical Guidelines of the 1975 Declaration of Helsinki.

## Inclusion and exclusion criteria

Main inclusion criteria were the presence of an active HCV infection and the indication to perform an antiviral therapy with interferon free DAA regimens for a compensated cirrhosis (Child-Turcotte-Pugh CTP score A or B and MELD score <25) and advanced fibrosis (liver biopsy Metavir fibrosis score F3 or F4 and/or liver stiffness measurement with transient elastography ≥ 10 kPascal) on the basis of the prescribing information from Italian Association for the Study of the Liver (AISF) and the reimbursement policies of the Italian Drugs Agency (AIFA)(AISF/AIFA criteria number 1 and 4) [24]. Exclusion criteria were: indications for HCV DAA therapy in addition to those in the inclusion criteria (e.g. liver and other organs transplantation recipients, lymphoproliferative disorders and mixed cryoglobulinaemia, decompensated cirrhosis in liver transplant list etc.) and/or other causes of liver disease such as HBV co-infection, HIV, CMV, HSV, autoimmune diseases (primary biliary cirrhosis, autoimmune hepatitis), metabolic disorders involving the liver (Wilson disease, primary and secondary hemochromatosis), unsafe alcohol consumption (more than 30 g/day of alcohol for men and 20 g/day units for women), active tumors (liver and non-liver), active drug abuse in the past six months, active systemic infections. Patients with previous hepatocellular carcinoma in remission after locoregional and/or surgery ablation were included as a part of the number one criterion.

## Antiviral therapy

In this observational study, the patients included in the present study were treated, and followed-up, on the basis of the physician choices. It was only mandatory to them to adhere to the above cited prescribing information and reimbursement policies. In this way the patients received a DAA regimen containing Sofosbuvir (SOF) plus Ribavirin (RBV) or SOF plus Daclatasvir ± RBV or SOF plus Simeprevir ± RBV or Ombitasvir plus Paritaprevir plus Ritonavir ± Dasabuvir (3D) ± RBV or SOF plus Ledipasvir ± RBV on the

basis of the good clinical practice behaviors of the physicians participating in the present study [25]. We evaluated the response to antiviral treatment for all patients with universally accepted criteria. In particular, we defined an end of treatment (EoT) response when we found an undetectable HCVRNA at the end of the therapy. For the evaluation of sustained virological response (SVR) we performed HCVRNA assay at 12 weeks (or more) after EoT (SVR12). Patients were eventually defined as “relapsers”(RL) if, after achieving an EoT response, they were found to have positive HCVRNA at 12 weeks (or more) after the treatment. Non-responders (NR) were defined as patients with no HCVRNA clearance during the treatment. Virological breakthrough was defined as the reappearance of HCV RNA in patients with initial negativity during treatment. Drop-out was defined when a patient withdrew from the course of therapy because of adverse events and/or non-compliance.

## Liver stiffness and fibrosis scores measurements

Liver stiffness measurement (LSM) by TE was obtained from each patient included in the present study and at the end of therapy (EoT) as well as at least 12 weeks after EoT, at the time of SVR12 evaluation. The liver stiffness measurement was achieved by performing a transient elastography (TE) with a Fibroscan 502 device (Fibroscan Ecosense, Paris, France) [15]. The measurement was performed by two skilled operators (with an inter-observer $k>0.8$) on the basis of the recommendations of the manufacturer and the current guidelines. Particularly, LSM was performed after at least 6 hours of fasting with an intercostal approach on the $4^{th}$ right intercostal space. At least 10 valid consecutive measurements were made. The median of these measurements was considered the value of Liver Stiffness. TE exams were considered reliable if there was an interquartile range (IQR) <30% of the mean value and a success rate of at least 60% of the measurements [26].

APRI and FIB4 were calculated at the three time-points of laboratory evaluations (Baseline, EoT, SVR) and interpreted on the basis of the EASL guidelines indications for non-invasive assessment of fibrosis [26].

## Laboratory and clinical parameters

For every patient a complete clinical and laboratory assessment with routine biochemistry tests was performed at any time-point of this study (Baseline, EoT, SVR).

## Varices Assessment

Any patient who had an available EGD within 12 months the baseline evaluation was recorded. Varices were considered “large esophageal varices” (LEV) if were≥ grade 2 according to the recent guidelines [27].

## Statistical analysis

Collected data were evaluated by performing parametric and non-parametric tests when appropriate. In particular, Student’s t-test, Mann-Whitney U test, ANOVA and linear correlation were performed to compare continuous variables, and chi-square test with Yates correction or Fisher-exact test were used to compare categorical variables. To assess if continuous variables were normally or not normally distributed we performed a Kolgoromov-Smirnov K-S “Goodness of fit” test for normality, before applying the correct analysis. Multivariate analyses were performed by carrying out binary logistic regressions. Statistical

analyses were performed using the Statistical Program for Social Sciences (SPSS®) ver.16.0 for Macintosh® (SPSS Inc., Chicago, Illinois, USA). Statistical significance was defined when “p<0,05” in a two-tailed test with a 95% confidence interval.

# Results

## Patients

The study protocol is presented in supplementary figure s1: 815 patients were screened and 749 were enrolled. All the data here presented are to be intended by an Intention to treat (ITT) analysis.

Demographical and clinical data of the patients included in the study are presented in table 1. Five hundred and thirteen patients out of 749 (69.5%) were enrolled as “F4” (i.e. compensated cirrhosis and/or LSM=>12.5 KPascal and/or eradicated HCC) and 236 (30.5%) as “F3” (LSM>10 and <12.5). No differences in age, BMI, sex and HCV genotypes distribution were found, whereas there was a statistically significant higher prevalence of diabetes in “F4” patients if compared to F3. With respect to the 513 F4 patients, 474 (86.9%) were CHILD A at the baseline (time of enrollment) and 71 (13.1%) were CHILD B. Patients with an HCC history in remission included in F4 population were 3.7% (19 pts), which is a slightly lower prevalence as those reported in other studies [28,29]. An HCC recurrence occurred in 4 of these 19 patients (21%) within the time of follow-up (12 weeks after EoT). A “de novo” HCC occurrence, intended as a first diagnosis of HCC with universally accepted criteria [30,31], was found in 4 out of 513 (0.78%) “F4” patients within the same short period of follow-up. Our findings on HCC occurrence and recurrence are lower than those found on the same subject in recent reports on DAA therapy [28,29]. F4 patients who have performed an esophagogastroduodenoscopy (EGD) within 48 weeks before starting the treatment were 169 (32.9%) and, of these, 118 (23.0%) were found to have Esophageal Varices (EV), of whom 25 had large esophageal varices (LEV).

## Antiviral therapy results

As reported in methods section, this was an observational study and the aim was not to evaluate the efficacy of DAA therapies on viral eradication, nevertheless we report an overall SVR rate of 97.3%, with a 98.7% of F3 and a 96.9% of F4 patients who reached the sustained response at 12 weeks after the end of the treatment. More precisely, SVR rate of Child A and Child B patients was 97.3% and 78.1%, respectively, which is in line with previous reports. In the present series the 2.7% of overall patients who did not achieved SVR were all relapsers.

## Laboratory parameters early improved at viral eradication

We collected laboratory data and clinical scores at the time of enrollment (baseline), at the end of treatment (EoT) and at the time of SVR (sustained virological response) of every patient who participated the study. Data collected in overall population, compared with each other, are reported in Table 2. Of note, there was a significant improvement between baseline and EoT for AST/ALT, GGT, ALP, bilirubin, creatinin, glycaemia, platelet count, and alfafetoprotein, whereas no significant improvements were caught between EoT and SVR. Findings were similarly found when we separately performed the same analysis in “F3” and “F4” patients (supplementary material table S1). Particularly, in “F4” patients the liver disease scores (CTP and

MELD) did not significantly differ at the various time-points, whereas the laboratory parameters AST/ALT, GGT, ALP, bilirubin, PLT and alfa-fetoprotein, demonstrated a significant improvement at EoT in comparison to baseline, with no further improvement at SVR.

## Indirect markers of fibrosis early improve after virological response

Similarly to what observed for laboratory parameters, indirect markers of liver fibrosis APRI and FIB4 significantly decreased at EoT with no further improvement at SVR12, with the exception of APRI in F4 patients which also improved at SVR12 (table 2 and supplementary table s2 and figures s2 and s3).

## LSM improved as early as EoT was achieved

In SVR patients, LSM improved from a baseline mean value of 19.3 (±11.2) to 15,2 (±9,7) at the end of therapy ($p<0.001$), with no further improvement at SVR12 (14.2 ±11.7) (p:0.519) (table 2). Also when we analyzed only F4 patients we found that LSM improved from a mean baseline value of 24.7 (±11.5) to an EoT value of 18.0 (±10.1) ($p<0.001$) without any significant improvement between EoT and SVR (supplementary table S1 and figure 1 panel B). As expected, no improvement from the baseline was found at EoT and SVR12 in relapsers patients (Figure 1 panel C). Interestingly, no significant improvement in LSM was found in type 2 diabetes mellitus affected patients that achieved SVR (figure 1 panel D).

## Factors influencing LSM improvement

We performed a univariate and ~~a~~ multiple logistic regression with the aim to find if there were any factors influencing a significant improvement in LSM (at the EoT and/or SVR12). We defined as “significant improvement” in LSM a reduction equal or more than 2 KPascal for patients up to 13,9 kPascal, and 6 kPascal for Patients with a value equal or more than 14 Kpascal at the baseline evaluation. These values were chosen on the basis of their hypothetical clinical impact on patients: indeed, 2 KPascal is the measurement that is able to discriminate classes of fibrosis for patients up to 13 kPascal, and 6 kPascal may be considered a significant improvement in patients with a baseline value more than 13,9 kPascal in terms of necroinflammation, portal hypertension and fibrosis.

The univariate analysis of clinical and laboratory factors possibly influencing a significant LSM improvement is reported in table 3. As it can be noted, among the chosen parameters, low ALT, high blood glucose, type 2 diabetes mellitus and low platelets count were negatively associated to LSM improvement. At the consequent multivariable binary logistic regression, only diabetes, low ALT and low platelet count confirmed to be independently, and inversely, associated to LSM improvement (table 3).

## Portal hypertension features seem to influence LSM improvement after viral eradication

Results are reported in table 4. In the 169 patients with an available recent EV evaluation, the univariate analysis confirmed that ALT levels, glucose, type 2 diabetes and low platelets and the presence of any degree varices predicted a lack of LSM improvement after viral eradication. At the multivariable binary logistic regression only low ALT, diabetes and EV were independently associated with no LSM improvement.

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## Discussion

Current Interferon free DAA therapy has radically changed the viral eradication opportunities for HCV infected patients [25]. These, therapies, because of their great efficacy and safety gave the physicians the possibility to expand the indications to treat. Nowadays, also advanced liver diseases, that were previously excluded from treatment, or treated with very low efficacy and/or safety [2], achieve a viral eradication that is very often above 90%, also in decompensated diseases [3]. Western countries drug regulatory institutions, due to pharma-economy reasons and priority needs, precisely indicated the advanced stages of the disease as those with high priority to treatment (namely “F3” and “F4” liver diseases) [25]. The method to assess liver fibrosis has been largely committed to the liver stiffness measurement by transient elastography (TE) technique [15]. Italian regulations on DAA reimbursement, issued by the Italian drug agency (AIFA) together with Italian association for the study of the liver (AISF), indicated TE measurements of 10 and 12.5 kPascal as indicative respectively of F3 and F4 liver fibrosis by Metavir scoring system [24]. Nevertheless, mounting evidences indicates that TE in CHC does not exclusively measures liver fibrosis, but also portal hypertension and necroinflammation [20–23]. Based on that, together with the issues regarding the difference between a viral eradication and a “clinical response” to antiviral therapies, there is still debate on the clinical impact of the viral eradication on the supposed improvement of CHC, in particular in the advanced stage liver diseases [3]. In the present study we investigated, on a large “real-life” cohort of “F3” and “F4” patients coming to four tertiary centers for the cure of HCV in Southern Italy, the impact on clinical parameters and liver stiffness measurements of viral eradication. Moreover, we also aimed our study to find baseline characteristics that might eventually influence the liver stiffness and/or the clinical improvement after virus clearance. In our cohort, 69,5% of patients was F4 (86,9% with Child-Pugh stage A and 13,1% Child B) and 30,5% was F3. The differences between baseline and EoT laboratory tests, fibrosis indirect scores (APRI, FIB4) and LSM suggest an early clinical improvement after the occurrence of the serum virological clearance. No changes were noticed between EoT and SVR12 and that seems to be true both in F3 and F4 patients. Nevertheless, some patients, even achieving a sustained response, don’t improve their clinical performance (LSM). Questions arise as to whether or not other (co)-factors may influence the LSM measurements and, secondly, as to whether or not the viral clearance corresponds to a real clinical improvement. Univariate and a multivariate analysis, aimed to enlighten possible independent factors probably influencing a “significant improvement” in liver stiffness measurements, were performed. ALT values, T2DM presence and low platelet count negatively, and independently, influenced LSM measurements. These results support the idea that diabetes is confirmed to be a “bad trip companion” of HCV as it is already suspected by the high prevalence of diabetes in HCV patients [32], and that the higher is the liver necroinflammation before starting the treatment the better will be the clinical outcome. Of particular interest was the result related to the low PLT count. The main clinical reason for low platelets count in cirrhosis may be considered the spleen enlargement, that may be considered an indirect marker of portal hypertension. In this regard, the idea of a significant interference on clinical outcome in SVR patients was then considered. In order to test this hypothesis, we designed univariate and multivariate analyses in patients in whom a recent EGD was available and considered the presence of varices as an independent valuable variable. The presence of varices independently influenced LSM measurement. All these data seem to indicate that the presence of higher levels of necroinflammation prior to starting the treatment predict an early improvement in LSM values

and, on the contrary, varices (read portal hypertension) and/or diabetes seem to negatively influence LSM after achieving a viral clearance. These results are reasonably explained by the abovementioned evidence that transient elastography is considered to be influenced not only by liver fibrosis but also by necroinflammation (which is rapidly reduced by the viral eradication) and portal hypertension. By contrast, T2DM presence as a negative predictive factor may be accounted for the presence of a larger amount of fibrosis, due to the well-known role of T2DM on promoting fibrogenesis [33,34] or, alternatively, steatogenesis [35,36]. An element of great interest is coming from our data: that the early improvement of laboratory values and LSM seems to be found at EoT precisely in the majority patients who will subsequently achieve the sustained virological response In this way, this finding could be an useful tool to predict which patients will experience, with a good accuracy, a SVR. Inversely, the patients who will not show this “early improvement” will be, most likely, those who relapse, not achieving a SVR, or, alternatively, those who have portal hypertension and/or diabetes. These last patients are those who need a more careful follow-up, in a longer period of time, in order to understand if they will never improve their clinical parameters or, otherwise, they will improve more slowly.

Lastly, it has to be noticed that, in our cohort, we didn’t experience an increase in HCC occurrence and/or recurrence following DAA therapies, differently from some reports coming from other settings [28,29] and in line with other authors communications [37–40].

The present study has some limitations that are mainly due to its non interventional observational nature. The follow-up period is limited to twelve weeks after the end of the therapy and for this reason further evaluations are to be warranted within a longer period of time. Moreover, on regards of the hypothesis that portal hypertension may influence the improvement of liver stiffness measurements, the present study, due to its observational nature, did not directly evaluated it by measuring hepatic vein pressure gradient (HVPG), therefore these hypotheses are to be verified in further studies that carry out this type of analysis.

## Conclusions

The data here reported seem to confirm that the viral eradication leads to an early improvement of clinical, laboratory and indirect markers of liver fibrosis, and this can be detected as early as EoT is achieved, remaining stable at the sustained virological response.

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### Author contributions

MP, VR, AA, FM, AF, MD, NC, MM: participated to study conception and design, data analysis and interpretation, article drafting and revising it critically for important intellectual content, and gave final approval for publication.

DP, MC, AD, VC, EC: participated to study conception and design, article revising critically for important intellectual content, data collection, and gave final approval for publication.

### Conflict of interests statement

The authors declare that they haven’t any conflict of interests regarding the object of the present paper.

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## Legends to figures

**Figure 1:** Liver stiffness measurements at the three time-points of the study; **Panel A**: SVR patients overall; **Panel B:** SVR F4 patients; **Panel C:** relapser patients. **Panel D:** SVR diabetic patients.

**Table 1.**Demographic characteristics of patients.

| | **All patients** | **"F3" patients** | **"F4" patients** | ***P*value** |
|---|---|---|---|---|
| **No. of patients** (%) | 749 | 236 (31.5%) | 513 (69.5%) | |
| **Age** [Mean (±SD)] | 64.9 (±9.1) | 64.0 (±10.1) | 65.1 (±9.0) | 0.975 |
| **Male Sex** [n. (%)] | 428 (57.1%) | 134 (56.6%) | 288 (56.2%) | 0.985 |
| **BMI [**Mean (±SD)] | 25.9 (±3.7) | 25.5 (±3.0) | 26.1 (±3.9) | 0.147 |
| **Diabetes** [n (%)] | **105 (14.0%)** | **16 (6.6%)** | **88 (17.2%)** | **0.003** |
| **MELD** [Mean (±SD)] | - | - | 9.1 (±3.1) | |
| **Child** [Mean (A/B %)] | - | - | 6 (86.9/13.1) | |
| **Pre-tx HCC** [n(%)] | **19** (2.5%) | **0** | **19** (3.7%) | **0.019** |
| **HCC recurrences** [n(%)] | 4 (0.5%) | - | 4 (0.8%) | |
| **HCC Occurences** [n(%)] | 7 (0.9%) | 0 | 7 (1.36) | |
| **Hepatic Events** [n(%)] [(1)] | 3 | 0 | 3 | |
| **Genotypes (%)** | | | | 0.850 |
| **1b** | 62.9 | 64.7 | 67.2 | |
| **1a** | 4.0 | 4.2 | 4.8 | |
| **2** | 23.9 | 20.2 | 20 | |
| **3** | 6.8 | 5.9 | 5.9 | |
| **4** | 2.1 | 5 | 2.1 | |
| **Esophageal varices** [n (%)] | | **0** | **118(23.0)** | **<0.001** |
| **ETR** [n (%)] | 746 (99.6%) | 236 (100%) | 510 (99.4%) | |
| **SVR** [n (%)] | 730 (97.5%) | 233 (98.7%) | 497 (96.9%) | 0.068 |
| | | | | |

[(1)] Events: Ascites (2) . Esophageal or upper gastrointestinal bleeding (0). Porto-systemic encephalopathy (1)

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**Table 2**: Evaluation of clinical and laboratory parameters at baseline, end of therapy and at the time of SVR in all patients.

| **Overall patients n749** | | | | | |
|---|---|---|---|---|---|
| **Variable Mean** (±SD) | **Baseline** | **EoT** | **p** | **SVR** | **p** |
| **AST** U/L | **56.02(±56)** | **15.2 (±15.2)** | **<0.001** | 10.7(±10.7) | 0.681 |
| **ALT** U/L | **89.23 (±67.8)** | **22.7 (±19.9)** | **<0.001** | 22.2(±12.8) | 0.290 |
| **γGT** U/L | **44.39(±142.4)** | **35.4(±57.1)** | **0.001** | 32.9(±25.6) | 0.359 |
| **ALP** U/L | **134.9(±78.3)** | **120.2(±68.1)** | **0.005** | **107.8(±55.4)** | **<0.001** |
| **Bilirubin** mg/dL | **1(±0.75)** | **0.5(±0.74)** | **<0.001** | **0.8(±0.6)** | **0.014** |
| **Albumin** g/dL | 3.9(±2.7) | 3.9(±0.4) | 0.559 | 4.0(±0.3) | 0.455 |
| **Creatinin** mg/dL | **0.88(±0.3)** | **0.85(±0.3)** | **0.013** | 0.82(±0.2) | 0.817 |
| **Glycemia** mg/dL | **110(±33)** | **107(±35)** | **0.092** | 106(±30) | 0.890 |
| **Platelets** 10E3/μL | **140(±74)** | **159(±79)** | **<0.001** | **143**(±75) | **0.013** |
| **INR** value | 1.1(±0.2) | 1.1(±0.7) | 0.315 | 1.1(±0.1) | 0.455 |
| **Α-fetoprotein** ng/mL | **10.4(±12.7)** | **5.7(±10)** | **<0.001** | 7(±16) | 0.788 |
| **Cholesterol** mg/dL | **148(±40)** | **166(±36)** | **0.008** | 176(±34) | 0.383 |
| **HDL** mg/dL | 49(±13) | 50(±14) | 0.343 | 51(±11) | 0.245 |
| **FIB4** | **5.4(±4.9)** | **3.6(±9.3)** | **<0.001** | 3.3(±3.1) | 0.201 |
| **APRI** | **1.9(±2.2)** | **0.6(±1.5)** | **<0.001** | 0.6(±0.5) | 0.500 |
| **LMS** kPas | **19.3(±11.2)** | **15.2(±9.7)** | **<0.001** | 14.2(±11.7) | 0.519 |
| | | | | | |

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**Table 3.** Overall patients: Univariate analysis (Pearson Chi-Square. except BMI*. AGE* and ALT* - Spearman rho analysis) and multivariate (binary logistic regression) of baseline independent variables influencing a significant LSM improvement

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (CI) | p | OR (CI) | p |
| **Age*** | na | 0.144 | 0.964 (0.903-1.028) | 0.964 |
| **SEX M/F** | 1.38 (0.86-1.23) | 0.277 | 3.488 (0.387-29.624) | 0.270 |
| **BMI*** | na | 0.281 | 0.817 (0.614-1.086) | 0.164 |
| **Genotypes** | 1.83 (0.81-4.12) | 0.143 | - | - |
| **HCVRNA** | 1.0 (0.75-1.35) | 0.848 | - | - |
| **CHILD** | 1.17 (0.65-2.08) | 0.592 | 1.694 (0.338-8.491) | 0.522 |
| **MELD** | 0.903 (0.77-1.04) | 0.903 | 0.954 (0.645-1.412) | 0.815 |
| **Low ALT*** | **na** | **0.0007** | **0.988 (0.978-0.998)** | **0.025** |
| **Albumin** | 2.155 (0.688-6.751) | 0.187 | - | - |
| **Glucose** | **0.975 (0.956-0.993)** | **0.003** | 0.912 (0.661-1.075) | 0.116 |
| **Type 2 Diabetes** | **0.981 (0.886-0.995)** | **0.001** | **0.036 (0.004-0.324)** | **0.003** |
| **Low PLT** | **0.560 (0.547-0.788)** | **0.0001** | **0.975 (0.953-0.997)** | **0.023** |

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**Table 4.** Patients with EGD: Univariate analysis of baseline independent variables predicting a significative LSM improvement (Pearson Chi-Square. except BMI*. AGE* and ALT* - Spearman rho analysis

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | **OR (CI)** | **p** | **OR (CI)** | **p** |
| **Age*** | na | 0.140 | 0.962 (0.901-1.020) | 0.261 |
| **SEX M/F** | 1.37 (0.85-1.43) | 0.276 | 3.187 (0.356-25.620) | 0.255 |
| **BMI*** | na | 0.286 | 0.808 (0.604-1.088) | 0.155 |
| **Genotypes** | 1.73 (0.81-3.15) | 0.154 | - | - |
| **HCVRNA** | 1.1 (0.77-1.44) | 0.859 | - | - |
| **CHILD** | 1.37 (0.67-1.98) | 0.602 | 1.685 (0.347-7.451) | 0.543 |
| **MELD** | 0.913 (0.74-1.22) | 0.963 | 0.964 (0.605-1.514) | 0.807 |
| **Low ALT*** | **na** | **0.002** | **0.980 (0.978-0.9989** | **0.024** |
| **Albumin** | 2.143 (0.677-6.654) | 0.207 | - | - |
| **Glucose** | **0.955 (0.946-0.994)** | **0.002** | 0.903 (0.671-1.066) | 0.110 |
| **Type 2 Diabetes** | **0.982 (0.885-0.996)** | **0.001** | **0.038 (0.005-0.335)** | **0.004** |
| **Low PLT** | **0.550 (0.536-0.798)** | **0.001** | 0.975 (0.903-1.057) | 0.224 |
| **Varices** | **0.574 (0.502-0.791)** | **0.0001** | **0.956 (0.899-0.966)** | **0.024** |

A

Overall SVR

p<0.0001

p:ns

kPascal

80
60
40
20
0

Baseline
EoT
SVR12

Time of Therapy

B

F4 SVR

p<0.0001

p:ns

kPascal

80
60
40
20
0

Baseline
EoT
SVR12

Time of Therapy

C

No SVR

ns

ns

kPascal

60
40
20
0

Baseline
Eot
SVR12

Time of Therapy

D

Diabetes

p:ns

p:ns

kPascal

50
40
30
20
10
0

Baseline
ETR
SVR12

Time of Therapy

**Table S1**: evaluation of clinical and laboratory parameters at baseline. end of therapy and at the time of SVR in F3 and F4 patients

| **F3 patients (n.236)** | | | | | |
|---|---|---|---|---|---|
| **Variable Mean** (±SD) | **Baseline** | **Eot** | **P** | **SVR** | **P** |
| **AST** U/L | **62.4(±48)** | **21.4(±6.5)** | **<0.001** | 23.3(±8.2) | 0.468 |
| **ALT** U/L | **86.2(±74)** | **19.8(±9.8)** | **<0.001** | 21.6(±12.8) | 0.954 |
| **γGT** U/L | **47.8(±50)** | **22.1(±9.5)** | **0.001** | 20.5(±8.7) | 0.837 |
| **ALP** U/L | 107(±79) | 95.4(±70) | 0.071 | 94.3(±65.5) | 0.457 |
| **Bilirubin** mg/dL | **0.8(±0.3)** | **0.5(±0.6)** | **<0.001** | 0.6(±0.3) | 0.127 |
| **Albumin** g/dL | 3.9(±0.4) | 4(±0.4) | 0.648 | 4.2(±0.3) | 0.650 |
| **Creatinin** mg/dL | 0.88(±0.2) | 0.84(±0.2) | 0.132 | 0.84(±0.15) | 0.765 |
| **Glycemia** mg/dL | 100(±19) | 98(±20) | 0.302 | 99(±28) | 0.402 |
| **Platelets** 10E3/μL | **180(±71)** | **193(±81)** | **0.013** | 184(±88) | 0.194 |
| **INR** | 1.1(±0.1) | 1.2(±0.2) | 0.384 | 1(±0.1) | 0.388 |
| **A-fetoprotein** ng/mL | 6(±7.5) | 2.4(±1.5) | **0.014** | 3.3(±2.3) | 0.240 |
| **Cholesterol** mg/dL | 165(±49.9) | 179(±35) | 0.204 | 171(±18) | 0.966 |
| **HDL** mg/dL | 46.2(±15.3) | 46.8(±10.7) | 0.818 | 46(±7.9) | 0.804 |
| **FIB4** | **2.7(±1.5)** | **1.8(±0.9)** | **<0.001** | 2.7(±4) | 0.196 |
| **APRI** | **1(±1.1)** | **0.3(±0.1)** | **<0.001** | 0.4(±0.5) | 0.244 |
| **LMS** kPas | **10.1(±1.7)** | **8(±2.7)** | **<0.001** | 7.2(±3.2) | 0.086 |
| | | | | | |
| **F4 patients (n.513)** | | | | | |
| **Variable Mean** (±SD) | **Baseline** | **Eot** | **P** | **SVR** | **P** |
| **AST** U/L | **87.8(±63)** | **28.2(±17)** | **<0.001** | 26.3(±12) | 0.756 |
| **ALT** U/L | **91.8(±62.8)** | **24.9(±24)** | **<0.001** | 23.6(±13) | 0.210 |
| **γGT** U/L | **85.5(±212)** | **40.5(±66)** | **0.010** | 36.5(±28) | 0.390 |
| **ALP** U/L | **146(±79)** | **130(±68)** | **0.021** | **109(±52)** | **0.001** |
| **Bilirubin** mg/dL | **1.1(±0.8)** | **0.7(±0.8)** | **<0.001** | 0.9(±0.7) | 0.067 |
| **Albumin** g/dL | 4.2(±1.1) | 3.9(±0.5) | 0.567 | 4.1(±0.4) | 0.353 |
| **Creatinin** mg/dL | 0.89(±0.5) | 0.87(±0.4) | 0.068 | 0.82(±0.21) | 0.732 |
| **Glycemia** mg/dL | 114(±35) | 111(±35) | 0.152 | 109(±33) | 0.432 |
| **Platelets** 10E3/μL | **129(±75)** | **144(±76)** | **0.008** | 138(±68) | 0.062 |
| **INR** | 1.1(±0.2) | 1.1(±0.3) | 0.484 | 1.1(±0.1) | 0.281 |
| **A-fetoprotein** ng/mL | **12.3(±13.4)** | **8.7(±6.8)** | **0.001** | 9(±7.5) | 0.860 |
| **Cholesterol** mg/dL | **144(±35.6)** | **160(±33)** | **0.017** | 167(±36) | 0.271 |
| **HDL** mg/dL | **48.6(±15.7)** | **55.2(±19.5)** | **0.382** | 50(±13.3) | 0.185 |
| **FIB4** | **6(±4.4)** | **4.4(±2.5)** | **<0.001** | 3.2(±2.1) | 0.101 |
| **APRI** value | **2.2(±2.3)** | **0.8(±1.8)** | **<0.001** | **0.6(±0.5)** | **0.036** |
| **LSM** kPas | **24.7(±11.5)** | **18(±10.1)** | **<0.001** | 16.5(±13) | 0.647 |
| **Child-Pugh** | 5.4(±0.8) | 5.3(±0.7) | 0.347 | 5.1(±0.5) | 0.347 |
| **MELD** | 8.7(±2.2) | 8.6(±2.3) | 0.826 | 8.4(±2.0) | 0.169 |

**Legends to figures:**
**Figure s1:** Flow-chart of the present study protocol.

**Fig s2:** APRI test in F4 patients at the three time-points of the study.

**Figure s3:** FIB4 in F4 patients at the three time-points of the study.

815 Screened Patients

62 Excluded Patients
- 2 OLT recipients
- 5 OLT listed
- 26 HBV coinfection
- 9 HIV coinfection
- 7 LNH
- 12 Cryoglobulinaemia

4 No Consent

749 Patients enrolled (Baseline)

2 Voluntary discontinuation

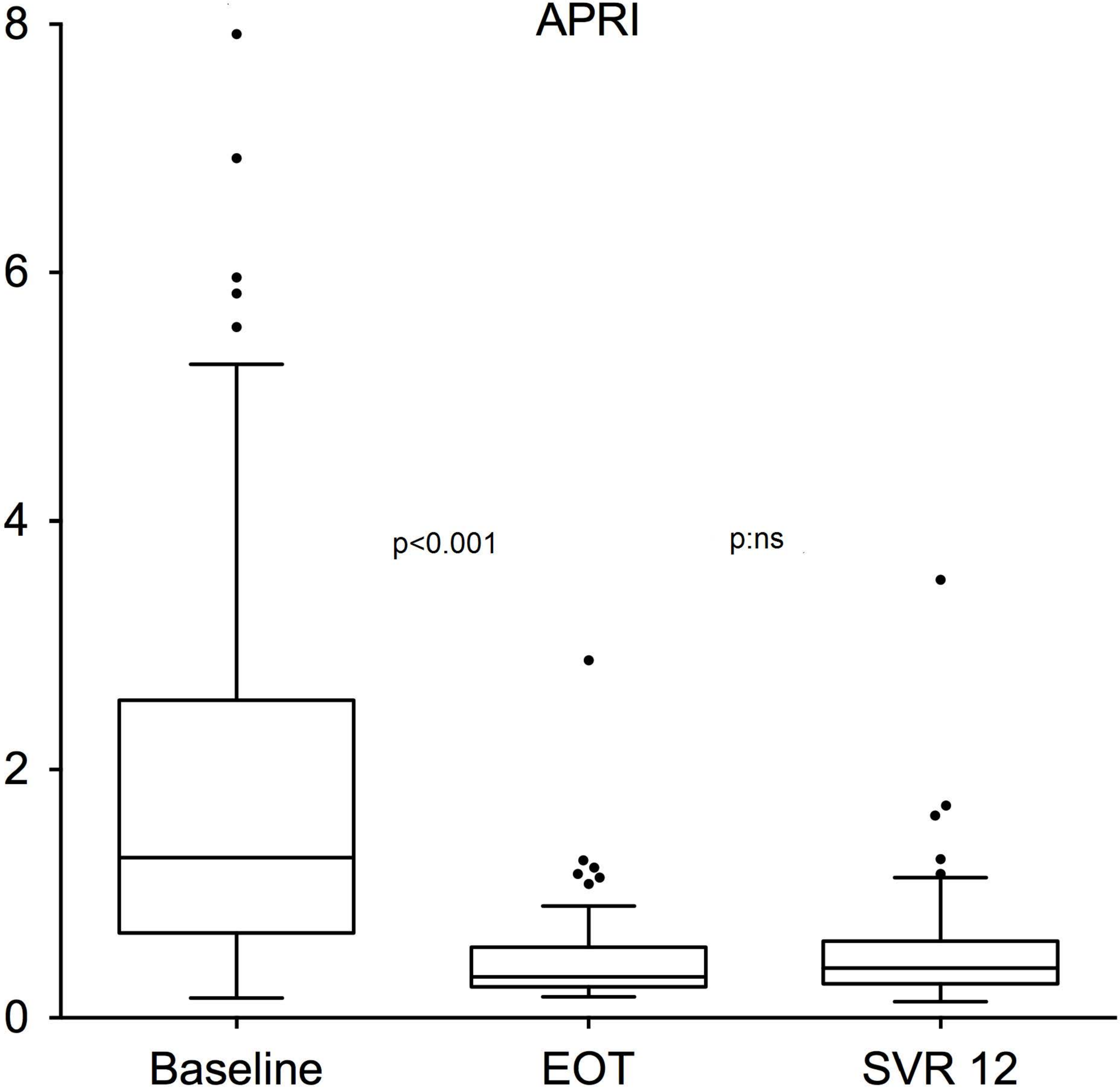
747 End of Treatment (EoT)

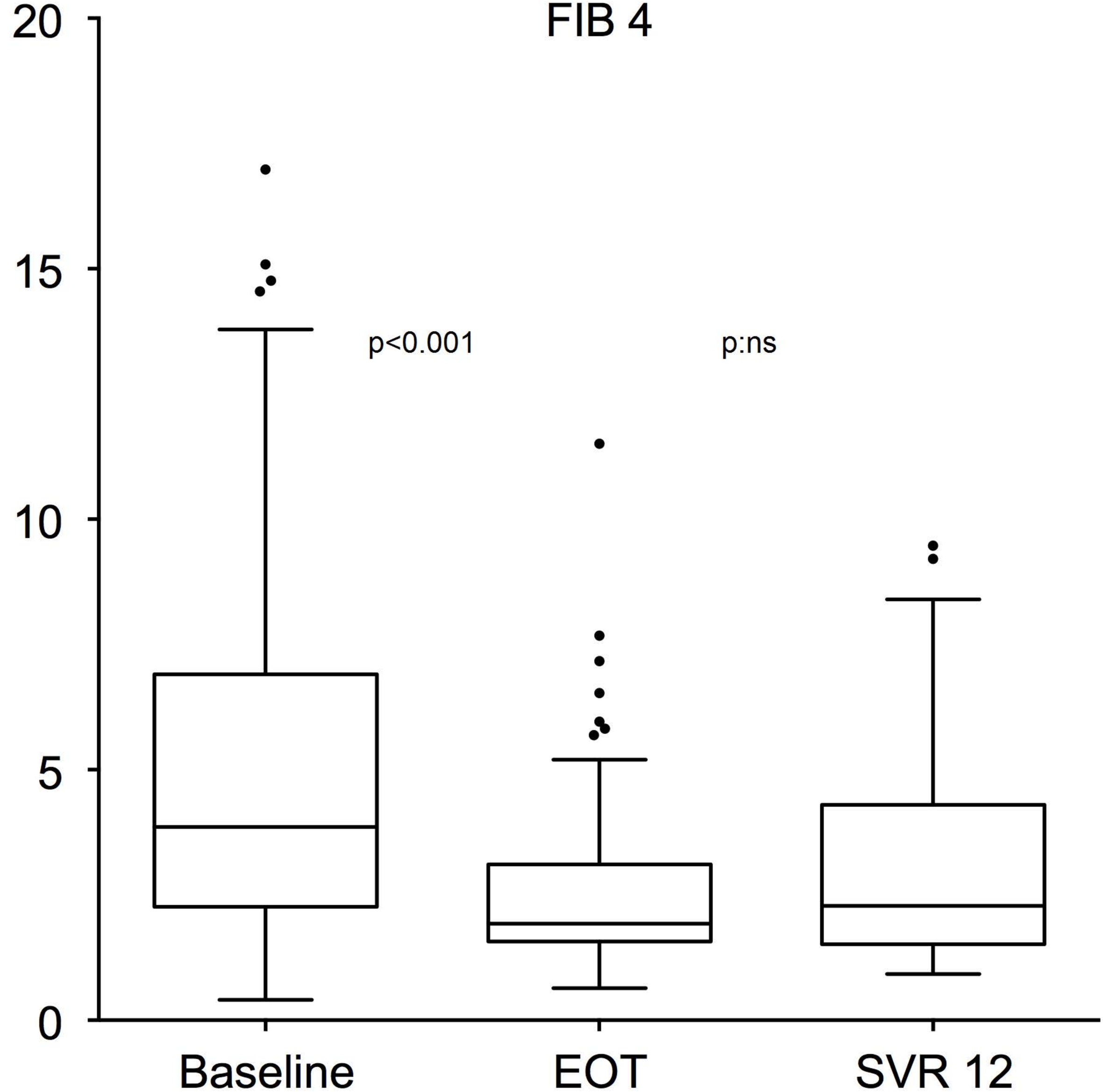
12 Failed to perform fibroscan

2 Liver unrelated deaths *

733 12 Weeks Follow-up (SVR12)

* 1 Miocardial infarction and 1 street accident after EoT


APRI
8
6
4
2
0
p<0.001
p:ns
Baseline
EOT
SVR 12


FIB 4
20
15
10
5
0
p<0.001
p:ns
Baseline
EOT
SVR 12