CONFLICT OF INTEREST: NONE DECLARED

ORIGINAL PAPER

Indirect Serum Fibrosis Markers in Hepatitis C Virus (HCV) Infection

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Assessment of liver fibrosis is important for making treatment decisions, as well as for predicting prognosis and therapeutic outcome in patients on chronic hemodialysis (HD) treatment and infected with hepatitis C virus (HCV). **The aim** of the present investigation was to evaluate changes in standard laboratory tests (AST, ALT, γGT, cholesterol and platelet count) and indirect serum fibrosis markers: AST-to-platelet ratio index (APRI), FIB-4 and Forns index, in chronically HCV-infected patients on maintenance HD with and without antiviral treatment. **Patients and methods:** A total of 38 patients on chronic HD program more than 3 months and with chronic hepatitis C, were included in the study. According to local legislature 14 patients receive antiviral therapy (24 or 48 weeks, according to HCV genotype) adjusted for patients on HD: eight of them achieved sustained virological response (SVR) and six did not. **Results:** All treated patients were HCV genotype 1. Baseline blood samples for standard laboratory tests and indirect serum fibrosis markers were collected on the day of antiviral treatment initiation, as well as at the end of follow-up treatment, 36 month later. At the beginning of antiviral treatment there were no significant differences in APRI, FIB-4, Forns and its components between patients who will achieve SVR and those who did not. A significant decrease of AST, ALT, γGT and APRI, and moderate decrease FIB-4 and Forns index was found at the end of follow-up in patients with SVR. In non-sustained responders group those three indexes and its components remained unchanged. Using cut-of values for APRI and FIB-4 (APRI<0,5 and FIB-4<1,45) it was registered that raised percentage of patients with "no fibrosis" at the end of follow-up in those who achieved SVR. Absence of fibrosis measured by Forns index remained unchanged in all groups of patients. **Conclusion:** Simple indexes as APRI and FIB-4, successfully decrease after antiviral treatment of chronic hepatitis C in hemodialysis patients. These parameters seems to be useful in monitoring for liver fibrosis rate after antiviral treatment in patients on maintenance HD infected by HCV and can be used for estimation liver fibrosis progression in candidates for cadaveric renal transplantation. Key words: HCV infection, Serum fibrosis markers, Antiviral treatment, APRI, FIB-4, Forns, Hemodialysis.

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1. INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% occurring in about 170 million infected persons (1). Patients on chronic hemodialysis (HD) are among the highest risk groups for the acquisition of HCV infection (2, 3). The annual incidence of HCV infection in this population ranges from 0.2-6.2%, which is approximately 100-1000 times higher than that in the general population (4). The reported prevalence rates of chronic hepatitis C among patients on HD ranges from 3.4-80% with great geographic variation (5). Most of patients in this population (65-92%) with acute hepatitis C become chronically infected (6), and some of them will develop serious conditions such as chronic active hepatitis, cirrhosis and hepatocellular carcinoma.

Recent studies have clearly shown that HCV-infected patients on maintenance HD are at increased risk of liver-related mortality (7, 8, 9). So far, antiviral treatment is useful and important for those patents. Identification of significant liver fibrosis stage is essential to establish the timing of antiviral treatment. Assessment of the effect of antiviral treatment on liver fibrosis is another desirable end point for evaluation of the efficacy of therapy (10). Although, liver biopsy is classically considered as ref-

erence standard to assess the extent of fibrosis, it has three major limitations: a risk of complications, sampling error and intra and inter observer variability (11). Patients on chronic HD are in high risk of complications, especially of bleeding and they are often reluctant to submit to repeated biopsies, which limits physician's ability to monitor disease progression and effects of treatment. Therefore, non-invasive assessment of liver fibrosis becomes important diagnostic tool for those patients. Several routine laboratory test combined in scores and indices such as Forns' score, AST to platelet ratio index (APRI) and FIB-4 index, have been developed and validated as useful non-invasive and inexpensive tools to detect significant fibrosis or cirrhosis accurately in clinical practice (12, 13, 14).

The aim of the present investigation was to evaluate changes in standard laboratory tests (AST, ALT, γGT, total cholesterol and platelet count) and indirect serum fibrosis markers: AST-to-platelet ratio index (APRI), FIB-4 and Forns index, in chronically HCV-infected patients on maintenance HD with and without antiviral treatment.

2. PATIENTS AND METHODS

2.1. Study population

A total of 38 patients on chronic hemodialysis program more than 3 months and with chronic hepatitis C, who were with detectable serum HCV-RNA level, not receiving any antiviral or antifibrotic therapy, were included in the study. Patients with other causes of chronic hepatitis (hepatitis B virus, chronic alcohol abuse, nonalcoholic steatohepatitis, and autoimmune liver disease) or they with thrombocytopenia, coinfection with hepatitis B virus or HIV, decompensated cirrhosis, neoplastic disease or poorly controlled autoimmune disease, were excluded from study.

Out of a total of 38 patients, 23 were men (60%), mean age 52.8±11.2 years and 15 women (40%), mean age 53.1±15.1 years. The patients were mostly above 50 years of age (65.8%) with the predominant age interval of 51-60 years (42.1%). Out of various renal diseases influencing end stage renal disease (ESRD) onset, most of the

subjects had pyelonephritis and other interstitial nephropathies (23.4%) and glomerulonephritis (22.4%). The patients were recruited from Hemodialysis unit Clinic of Nephrology, Clinical Center Nis, Serbia, on March 2008 and followed next 36 months.

2.2. Methods

At the beginning of the study, anti-HCV seroprevalence was determined by 3rd generation ELISA assay, and after that, HCV infection was confirmed by RT-PCR technique and genotyping was performed by restriction fragment length polymorphism. According to local legislature, 24 patients did not met necessary criteria and not received antiviral treatment, but the remaining 14 patient receive antiviral therapy. Standard antiviral treatment was adjusted for patients on maintenance HD: monotherapy with pegylated interferon alfa-2a (135 μg/weekly) for 24 or 48 weeks, according to HCV genotype. All of treated patients were HCV genotype 1, eight of them achieved sustained virological response (SVR) and six did not. SVR was defined by undetectable serum HCV-RNA by qualitative polymerase chain reaction assay at the 24 weeks after the end of therapy. At the end of follow-up, patients with SVR is still remained RT-PCR negative.

Baseline blood samples were collected on the day of antiviral treatment initiation, as well as at the end of follow-up treatment. Laboratory test included platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (γGT) and total cholesterol. These parameters were used also to calculate Forn's score, APRI and FIB-4 index, at the baseline and 36 month later.

The APRI is a numerical value that is calculated using the following formula: APRI = [AST(IU/L)/upper limit of normal (IU/L)] x 100/platelets ($10^3/mm^3$). The FIB-4 index is also a numerical value that is calculated using the following formula: FIB-4 = Age x AST(IU/L)/[platelets ($10^3/mm^3$) x $ALT^{1/2}$ (IU/L)].

Patient characteristics	Mean/median (range/SD)
Age (yr)	52 (20-75)
Age at infection (yr)	49 (21-74)
Time of progression (yr)	6.41 (1-9)
AST (IU/L)	31.9 ± 16.7
ALT (IU/L)	56.9 ± 29.1
γGT (IU/L)	94.5 (14-381)
Chol (mmol/L)	4.18 ± 0.86
PLT ($10^3/mm^3$)	178.7 ± 63.3
FIB-4	1.196 (0.199-3.963)
APRI	0.513 (0.121-1.984)
Forns	6.075 ± 1.689

Table 1. Clinical and laboratory data of the study population. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma glutamyl transpeptidase; Chol, total cholesterol;

The Forns score is a numerical value that is calculated using following formula: Forns = 7.811 – 3.131 x Ln [platelets ($10^3/mm^3$)] + 0,781 x Ln [γGT(IU/L)] + 3.467 x Ln [age (yr)] – 0,014 x cholesterol (mmol/L).

2.3. Statistical methods

Student-t test was used for group comparisons (if there was normal distribution of frequencies within the groups) or non-parametric Mann-Whitney Rank Sum Test if the frequency distribution was non-normal. Statistically significant difference in

	Patients with SVR	Patients without SVR	p
AST (IU/L)	39.8 ± 20.5	28.3 ± 10.2	n.s.*
ALT (IU/L)	67.5 ± 29.5	71.5 ± 26.4	n.s.*
γGT (IU/L)	109.5	140.5	n.s.**
Chol (mmol/L)	3.80 ± 0.83	4.02 ± 0.75	n.s.*
PLT ($10^3/mm^3$)	179.1 ± 97.1	176.6 ± 64.6	n.s.*
FIB-4	1.382	0.850	n.s.**
APRI	0.879	0.521	n.s.**
Forns	6.541 ± 1.767	6.047 ± 1.066	n.s.*

Table 2. The baseline characteristics of the indirect serum fibrosis markers (APRI, FIB-4, and Forns) and its components in sustained virological responders and non-sustained responders group (*) t-test; (**) Mann-Whitney Rank Sum Test. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma glutamyl transpeptidase; Chol, total cholesterol;

	All patients on antiviral therapy	Patients with SVR (i)	Patients without SVR (ii)	p	Post test statistic
AST (IU/L)	31.9 ± 16.7	18.8 ± 6.6	29.2 ± 11.5	< 0.05[a]	i vs. ii, p<0.05[c]
ALT (IU/L)	56.9 ± 29.1	21.7 ± 13.3	48.2 ± 23.3	< 0.05[a]	i vs. ii, p<0.05[c]
γGT (IU/L)	94.5	45.0	73.6	< 0.05[b]	i vs. ii, p<0.05[d]
Chol (mmol/L)	4.18 ± 0.86	4.60 ± 0.48	3.99 ± 0.60	n.s.[a]	
PLT (10^3/mm^3)	178.7 ± 63.3	187.1 ± 50.2	164.8 ± 73.9	n.s.[a]	
FIB-4	1,196	0.976	1.128	n.s.[b]	
APRI	0.513	0.280	0.400	< 0.05[b]	i vs. ii, p<0.05[d]
Forns	6.075 ± 1.689	5.544 ± 1.071	6.626 ± 1.936	n.s.[a]	

Table 3. Changes in the mean indirect serum fibrosis markers (APRI, FIB-4 and Forns index) and its components during a 36 month follow-up period aANOVA (data are mean ± SD), bKruskal-Wallis One way ANOVA on ranks (data are median value), cTukey method for pairwise comparisons, dDunn's method for pairwise comparisons

one of the observed parameters among three or more groups of examinees was determined with variance analysis (ANOVA) or variance rank-analysis (Kruskal-Wallis ANOVA) in case of non-normal frequency distribution.

For the data with normal distribution of frequencies, mean value (x) and standard deviation (SD) were calculated, moreover, for non-normally distributed data, median, percentiles (C_{25} and C_{75}) and range (min. and max.) were calculated. Statistical significance was established for p values below 0.05. Analyses were performed using the SAS statistical package version 8e (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

A total of 38 patients met the inclusion criteria. The baseline characteristics of the subjects are described in Table 1. The median age at the time of initial analysis was 52 years (range: 20-75). Sixty percent (23/38) of patients were male, with mean age 52.8±11.2 years, and forty percent (15/38) were female, with mean age 53.1±15.1 years.

The baseline characteristics of the indirect serum fibrosis markers (APRI, FIB-4, and Forns) and its components in patients with SVR and those without SVR are summarized in Table 2. There were no significant differences in these parameters between sustained responders and non-sustained responders at the time of beginning antiviral treatment.

Changes in the mean indirect serum fibrosis markers APRI, FIB-4, Forns and its components during a 36 month follow-up period are summarized in Table 3. At the end of study a significant de-

crease of AST, ALT and γGT was observed in patients who achieved SVR

but remained unchanged in those who did not. A significantly decrease APRI, but not significantly decrease FIB-4 and Forns index was found at the end of follow up in patients with SVR, whereas those three indices remained unchanged in non-sustained responders.

The individual changes in indirect serum markers (APRI, FIB-4 and Forns) in patients with SVR, without SVR and patients who did not undergo antiviral treatment are shown in Figure 1. On an individual basis the APRI, FIB-4 and Forns indexes decreased in most sustained virological responders, but in only a minority of non-sustained virological responders and pa-
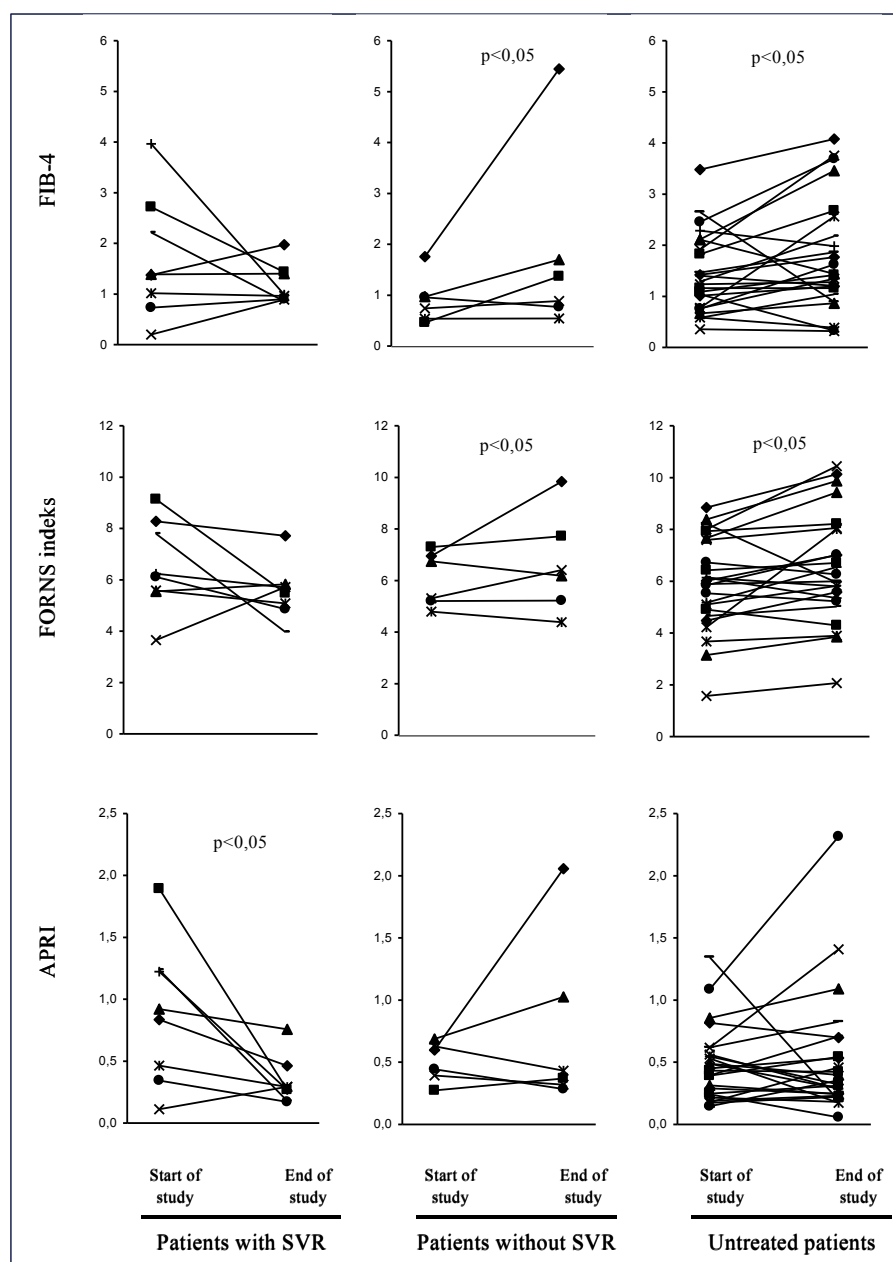

Figure 1. The individual changes in indirect serum indexes (APRI, Forns and FIB-4) between the start and end of study in the patients with SVR, without SVR and those without therapy
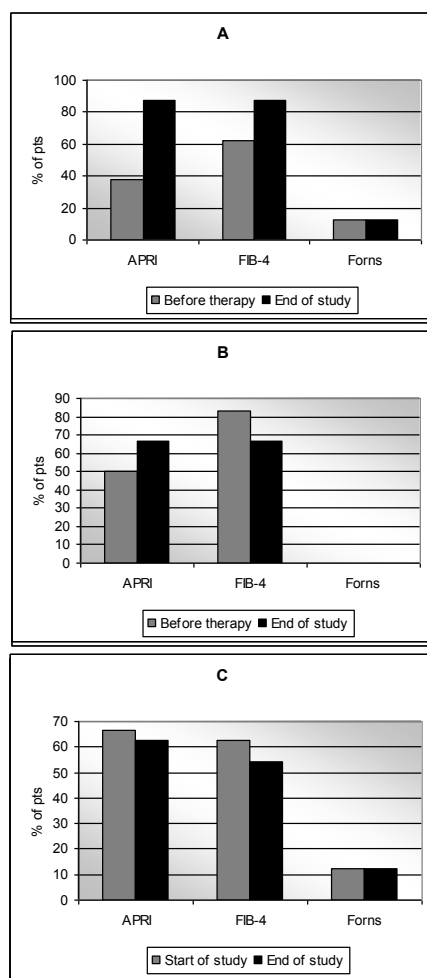
Figure 2. Changes in percentage of patient with "no fibrosis" according to the values of APRI, FIB-4 and Forns-indexes, in patients (A) with SVR, (B) without SVR and (C) those without antiviral therapy.

tients untreated with antiviral treatment, in most of whom the APRI, FIB-4 and Forns index remained unchanged or increased.

Changes in percentage of patients with absence of fibrosis between the baseline and the end of follow-up measured by APRI, FIB-4 and Forns indexes are shown in Figure 2. Using cut-off values for APRI and FIB-4 indexes (APRI less than 0,5 and FIB-4 less than 1.45), we registered that raised percentage of patients with "no fibrosis" (according to APRI index, from 37.5 to 87.5% and FIB-4 index, from 62.5 to 87.5%) at the end of follow-up in group of patients with SVR. Also it has been found that between the baseline and the end of follow-up decrease the percentage of patients with absence of fibrosis in non-sustained responders and untreated group of patients. Absence of fibrosis measured by Forns index remained un-

changed in all group of patients.

4. DISCUSSION

Patients on maintenance HD, candidates for renal transplantation have to be treated for hepatitis C before surgery, since HCV infection has a negative impact on graft and patients survival (15). Because of slow evolution of chronic hepatitis over several decades, it has been difficult to demonstrate that therapy prevents complications of liver disease. Accordingly, treatment responses are defined by surrogate biological parameters rather than by clinical end points (16). Because of limitations of liver biopsy (mainly sampling error and risk), several non-invasive biomarkers have recently been validated as alternatives for fibrosis staging, with diagnostic and prognostic values that are similar to those of biopsy (17, 18). A variety of noninvasive tests are proposed to estimate liver fibrosis in HCV patients with normal renal function, but a few data are available regarding the utility of those tests in patient on HD with HCV chronic infection. Several indirect markers of liver fibrosis as APRI, Forns and FIB-4 are simple, reproducible, readily available and able to follow disease progression have been proposed in recent years. In HCV positive patients on maintenance HD APRI identified significant fibrosis with accuracy of 80.1% and well correlated with the biopsy (19, 20, 21). Recent data show that simple serum fibrosis markers levels can decrease after successful antiviral treatment of chronic viral hepatitis C.

In our study we find out that serum cholesterol, platelet counts and particularly transaminases, which are not directly involved in hepatic fibrogenesis or fibrolysis, may change under antiviral therapy, mostly in sustained virological responders. These data was also confirmed in study of Martinez at al (10). Considerable post-treatment changes of Forns score, APRI and FIB-4 we demonstrated in our study: level of the APRI index was statistically significant lesser in patients with SVR, while FIB-4 and Forns index also decrease in those patients but not significantly. Possible explanation we can find in fact that Forns index and FIB-4 includes the parameter of patient age,

their score increases according to patient age and therefore may not accurately reflect improvement in fibrosis after treatment. In non-sustained responders and patients without antiviral treatment these indices remained unchanged. Similar results were found in several different studies in patients with normal renal function and patient on maintenance HD (10, 20, 22). According to APRI and FIB-4 cut-off values for excluding fibrosis (for APRI index, value les than 0,5 and for FIB-4 index, value les than 1.45) we registered at the end of follow-up that a proportion of patients with "no fibrosis" increase in patients who achieved SVR. At the same time and the same way, we analyzed non-sustained responders and patients without antiviral treatment and we found that decrease a percentage of patients with "no fibrosis".

In conclusion, simple novel indices as APRI and FIB-4, decrease after successful antiviral treatment of chronic hepatitis C in hemodialysis patients. This parameters seems to be useful in monitoring for liver fibrosis rate after antiviral treatment in patients on maintenance HD infected by HCV. Also these parameters can be used for estimation liver fibrosis progression in candidates for cadaveric renal transplantation. In addition, its applicability to the majority of patients and possibility to reduce the need for liver biopsy is very important and useful for patient on chronic HD. We need additional studies to support these findings.

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