DR. ELISA BILIOTTI (Orcid ID : 0000-0002-4540-1815)

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HCV cirrhotic patients treated with direct acting antivirals: detection of tubular dysfunction and resolution after viral clearance

Biliotti Elisa[1], Palazzo Donatella[1], Tinti Francesca[2], D'Alessandro Maria Domenica[3], Esvan Rozenn[1], Labriola Raffaella[3], Cappoli Andrea[2], Umbro Ilaria[2], Volpicelli Lorenzo[1], Bachetoni Alessandra[3], Villa Erica[4], Mitterhofer Anna Paola[2], Rucci Paola[5], Taliani Gloria[1].

1. Hepatology Unit, Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy.
2. Nephrology Unit, Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy.
3. Clinical Pathology, Department of Experimental Medicine, Sapienza University, Rome, Italy.
4. Division of Gastroenterology, Azienda Ospedaliero-Universitaria Policlinico di Modena, University of Modena and Reggio Emilia, Modena, Italy.
5. Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy.

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Corresponding Author:
Dr. Francesca Tinti
Sapienza University of Rome, viale dell'Università n°37, Rome, Italy
E-mail: francesca.tinti@uniroma1.it

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Abstract

Background/Aims

Hepatitis C virus (HCV) has been identified in tubular epithelial cells of infected patients, however the presence of tubular dysfunction, which is a risk factor for chronic kidney disease, has never been examined in vivo. The present prospective longitudinal study aimed to estimate the prevalence of tubular dysfunction alone or with glomerular damage and its evolution after HCV clearance in cirrhotic patients.

Methods

One-hundred-thirty-five consecutive Child-Pugh-A cirrhotic patients were evaluated before antiviral treatment and six months after the end of therapy. Tubular dysfunction was evaluated by urinary-alpha1-microglobulin-to-creatinine-ratio (α1-MCR), glomerular damage was assessed by urinary-albumin-to-creatinine-ratio (ACR).

Results

Almost all the patients (93.3%) showed a normal or mildly decreased e-GFR (KDIGO-G1/G2-categories). Tubular dysfunction was found in 23.7% (32/135) of patients, co-occurring with glomerular damage in 37.5% (12/32) of cases, while glomerular damage was found in 16.3% (22/135) of patients. In multiple logistic regression, glomerular damage and the concomitant presence of diabetes and hypertension were the only predictors significantly associated with tubular dysfunction. After HCV-clearance, patients experienced a significant reduction of α1-MCR levels (21.0 vs 10.5 μg/mg, p=0.009) and tubular dysfunction resolved in 57.1% of subjects.

Conclusions

Tubular dysfunction is an unrecognized feature of HCV-related kidney disease in cirrhotic patients and its presence should be primarily investigated in subjects with glomerular damage, diabetes and hypertension, despite normal e-GFR. Tubular dysfunction resolves in the majority of cases after HCV clearance, however, it may persist after antiviral treatment and further studies should evaluate its long term impact on kidney function.

Keywords: Glomerular damage, Proximal tubular dysfunction, Chronic hepatitis C, Direct acting antivirals (DAAs), Compensated cirrhosis

INTRODUCTION

Hepatitis C virus (HCV) infection is a systemic disease, associated with several extra-hepatic manifestations including chronic kidney disease (CKD) (1). Patients with chronic hepatitis C (CHC) have a 23% greater risk of developing CKD compared to uninfected individuals with a prevalence that ranges between 5% and 18% (2-8). Moreover, cirrhosis and other comorbidities contribute to the occurrence and evolution of CKD (9, 10).

Glomerular disease is a widely recognized feature of HCV related nephropathy (11-15), on the contrary, the association between HCV and tubular damage is a much less clarified issue, which has never been examined in vivo. The presence of HCV core protein, HCV-RNA genomic sequences and HCV-RNA replicative strand has been demonstrated in tubular epithelial cells of HCV patients by histological examination (16-18). This condition was associated with interstitial inflammatory cell infiltration and fibrosis (18), suggesting that HCV infection, as various types of viral infections (19, 20), may be a pathogenic factor of tubulointerstitial injury.

Increasing evidence shows that tubular damage is an important prognostic risk factor for the onset and the progression of CKD (21, 22). Recently, several urinary biomarkers of tubular damage

have been employed to predict renal dysfunction, among them, alpha1-microglobulin to creatinine ratio (α1-MCR) and kidney injury molecule 1 (KIM-1) have been widely used (23-25). Alpha1-MCR is a low molecular weight protein filtered by the glomerulus but fully reabsorbed by proximal tubular epithelial cells, therefore its presence in the urine is indicative of tubular dysfunction (24), while KIM-1 is a transmembrane protein produced by proximal tubular epithelial cells in the setting of injury, thus it is a reliable marker of early tubular damage (25).

Direct acting antiviral agents (DAAs) have revolutionized the HCV treatment landscape with very high cure rates (over 95%), excellent tolerability, short treatment duration (8-16 weeks) and low treatment failure (26, 27, 28). The effect of DAAs treatment on renal damage has not been completely clarified, since published studies report a partial benefit of viral eradication in patients with cryoglobulinemic glomerulonephritis (29, 30) but the effect of HCV clearance on tubular damage has never been examined.

The aims of the present prospective longitudinal study were to: 1) estimate the prevalence of tubular dysfunction alone or in association with glomerular damage in patients with HCV Child-Pugh A cirrhosis 2) evaluate the impact of HCV clearance on tubular dysfunction in subjects treated with DAAs.

MATERIALS AND METHODS

Patients

One hundred thirty-five consecutive Child-Pugh A cirrhotic patients receiving DAAs treatment for CHC from January 2015 to January 2016 were prospectively enrolled at the Liver Diseases Unit, Policlinico Umberto I, Rome. At that time, in Italy, DAA antiviral treatment could only be prescribed to subjects with advanced liver disease according to 2015 AIFA criteria (31).

The study protocol was approved by the Institutional Review Board (Protocol Number 3614) and all patients provided their written informed consent to participate in the study.

Patients were excluded if they had evidence of: 1) liver disease of different or mixed etiology (autoimmune hepatitis, alcoholic liver disease, hepatitis B, haemochromatosis, Wilson's disease, α1-antitrypsin deficiency); 2) decompensated cirrhosis (Child-Pugh classes B and C), according to reduce confounding factors for kidney disease such as hepato-renal syndrome or spontaneous bacterial peritonitis; 3) hepatocellular carcinoma (HCC); 4) HIV coinfection; 5) any malignancies; 6) new diagnosis of diabetes or decompensated diabetes in the six months before enrollment in the study, despite administration of oral antidiabetic medication and/or insulin (Haemoglobin A1c

higher than 7%), according to ESC/EASD guidelines (32); 7) new diagnosis of arterial hypertension or uncontrolled arterial hypertension despite administration of antihypertensive treatment (systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg), according to ESH/ESC guidelines (33).

Methods

Demographic, clinical, laboratory and virological data were collected before starting antiviral therapy (baseline, T0) and six months after the end of treatment (FU-6).

Laboratory data included complete blood count and standard biochemistry analyses (serum creatinine, blood urea nitrogen, albumin levels and liver function tests), HCV genotype and HCV-RNA viral load.

All patients were systematically tested for cryoglobulins at baseline with the following methods, as previously reported (34). Briefly, blood samples were collected into tubes prewarmed at 37°C, transported in a temperature-controlled flask, allowed to clot, and centrifuged at 37°C for serum separation. Sera were kept at 4°C for 7 days and cryocrit was expressed as the percentage of serum volume after centrifugation.

Hepato-splenic ultrasound and transient elastography (*Fibroscan*) were performed before starting treatment. The diagnosis of cirrhosis was done by liver biopsy and/or a value of liver stiffness ≥ 12.5 KPa (35).

Renal evaluation

Kidney function was evaluated by estimated glomerular filtration rate (e-GFR), which was calculated using the Chronic Kidney Disease Epidemiology Collaboration Formula (CKD-EPI-2009 formula) (36) at T0 and FU-6 and classified according to the Kidney Disease Improving Global Outcomes (KDIGO) Guidelines 2012 (37).

Tubular dysfunction and glomerular damage were evaluated at T0 and FU-6 by urine analysis.

Tubular dysfunction was defined by urinary α1-MCR values higher than 14 µg/mg. Resolution of tubular dysfunction was defined by α1-MCR normalization (<=14 µg/mg). In 52 consecutive enrolled patients, urinary quantification of KIM-1 was also performed at T0 and FU-6.

Glomerular damage was defined by urinary albumin to creatinine ratio (ACR) values higher than 30 mg/g. According to KDIGO Guidelines 2012, an ACR value between 30 and 300 mg/g corresponds to a moderate increase and an ACR value > 300 mg/g corresponds to a severe increase (37).

Resolution of glomerular damage was defined by normalization of ACR values (<=30 mg/g).

In order to monitor the impact of confounding factors on ACR evaluation, life style (diet, physical exercise, smoking), weight, blood pressure and drugs were recorded monthly during antiviral treatment and every three months during follow-up, while fasting plasma glucose and haemoglobin A1c were evaluated at T0, end of treatment (EOT) and FU-6 in diabetic subjects. In addition a self-recorded blood pressure was performed by all enrolled patients with arterial hypertension and was examined at each scheduled visit.

At least two measurements of α1-MCR, ACR and e-GFR values were performed at each time point (T0 and FU-6) in each patient.

Measurement of markers of renal damage

The second morning urine sample was collected, centrifuged and aliquoted.

The first aliquot was used for the routine urinary analysis on an automated analyser (Roche, Basel, Switzerland) to assess ACR, glomerular damage was defined by urinary ACR values higher than 30 mg/g according to KDIGO Guidelines 2012 (36).

The second aliquot was used for determining the urinary level of α1-microglobulin on a nephelometer analyzer (Siemens BN ProSpec, Germany). Data were expressed as α1-microglobulin/creatinine ratio (α1-MCR) to improve the practical estimation of excretion rate (38). The detectable limit of the α1-microglobulin assay was 5.56 mg/L. Intra-assay and inter-assay coefficients of variation (CVs) were 4.9% and 10.1%, respectively. In our study population the upper quartile values of α1-MCR was 14 μg/mg, corresponding to previously reported literature (39, 40). The mean value of α1-MCR, measured in spot urine samples of an internal control group of 33 healthy subjects (18M, 15F, mean age 52 years) with no clinical history of kidney diseases, diabetes, hypertension and inflammatory conditions, was 5.93 μg/mg (range 2.2-11.5 μg/mg). Therefore, proximal tubular dysfunction was defined as α1-MCR value > 14 μg/mg.

The third aliquot was frozen at -80°C until processed for KIM-1 measurement. The urinary levels of KIM-1 were measured with Quantikine ELISA kits (R&D Systems). The detection range of the kit was 0–10 ng /ml and the sensitivity was 0.009 ng/ml. The coefficients of variations (CV %) for intra-assay precision was less than 5%, and for inter-assay precision was less than 8%. The assay, which recognizes recombinant and natural human KIM-1, was used according to the manufacturer's directions. Urine concentration of KIM-1 was normalized to urine creatinine concentration to account for variations in urine concentrations. Serum creatinine was measured with an enzymatic creatinine assay (Roche Diagnostics GmbH, Mannheim, Germany).

HCV treatment

DAA regimen was chosen according to the Italian guidelines and to the availability of different regimens over time. Treatments included: 1) sofosbuvir and ribavirin 2) sofosbuvir and simeprevir with or without ribavirin 3) sofosbuvir and ledipasvir with or without ribavirin 4) paritaprevir/ritonavir, ombitasvir and dasabuvir with or without ribavirin 5) paritaprevir/ritonavir and ombitasvir with or without ribavirin 6) sofosbuvir and daclatasvir with or without ribavirin. The treatment duration varied from 12 to 24 weeks according to the infecting genotype and ribavirin use. Dose adjustments, treatment interruption and adherence were recorded.

Sustained virologic response (SVR) was defined as serum HCV-RNA undetectability 12 weeks after the end of treatment (FU-3).

Statistical analysis

Continuous variables were summarized as mean ± standard deviation or median ± interquartile range (IQR) and categorical data as counts and percentages.

Comparisons between groups were performed using χ^2 test or Fisher's exact test for categorical variables, and t-test or Mann-Whitney test for continuous variables. Changes from baseline in biochemical parameters were analyzed using paired-sample t-test or Wilcoxon test as appropriate.

Receiver operating characteristic (ROC) analysis was used to identify the optimal ACR cut-off as predictor of glomerular damage resolution that balanced sensitivity (SE) and specificity (SP).

Multiple logistic regression was used to identify the independent baseline predictors of proximal tubular dysfunction and of *de-novo* tubular dysfunction. The significance level for all analyses was set at $p < 0.05$.

Data were analysed using IBM SPSS, version 25.0 (SPSS Inc, Chicago, USA).

RESULTS

Patient characteristics

The baseline features of the 135 HCV Child-Pugh A cirrhotic patients are summarized in Table 1. Mean age was 62.6 ± 10.8 years, most of the patients were male (61.5%), 41.5% had previously diagnosed arterial hypertension stably controlled by antihypertensive treatment and 19.3% had controlled type-2 diabetes mellitus; 11.9% presented combined diabetes and arterial hypertension. Mean body-mass index (BMI) was on average 24.9 kg/m^2. Mean liver stiffness was 23.8 ± 13.9 kPa. The majority of patients were treatment-experienced (84/135, 62.2%). Genotype 1 was the

most frequent (89/135, 65.9%) and mean baseline HCV-RNA viral load was 5.8 ± 0.9 Log_{10} IU/ml. Cryoglobulins were detectable in 28.9 % of patients (39/135) with a mean cryocrit value of 3.4 ± 2.7%.

Baseline renal damage evaluation

Almost all the patients (126/135, 93.3%) showed a normal or nearly normal e-GFR (KDIGO G1 or G2 categories), while a moderate e-GFR decrease (G3a) was found in the remaining patients (9/135, 6.7%) (Table 1).

Thirty-two patients (23.7%) showed tubular dysfunction, which co-occurred with glomerular damage in 12 of them (37.5%). The median α1-MCR value of patients with tubular dysfunction was 21 (17.25-27.50) μg/mg.

Compared with patients without tubular dysfunction, those with tubular dysfunction were older (66 [59.2-75.7] vs 61 [53-69] years, Mann-Whitney test, p=0.020), had lower e-GFR values (84.5 [74.5-97.2] vs 93.0 [84.0-102.0] mL/min/1.73m², Mann-Whitney test, p=0.044), had more frequently a history of arterial hypertension (62.5% vs 34.9%, p=0.006) and diabetes (37.5% vs 13.6%, p=0.003) and had a higher prevalence of glomerular damage (37.5% vs 9.7%, p<0.001) (Table 2). In a multiple logistic regression predicting tubular dysfunction, of these 5 significant predictors, glomerular damage remained significant (OR=3.32, 95% CI 1.16-9.57, p=0.026), together with the concomitant presence of diabetes and hypertension (OR=4.23, 95% CI 1.10-16.27), while diabetes alone failed to achieve statistical significance (OR=3.95, 95% CI 0.84-18.60).

Urinary KIM-1 levels were examined in a subset of 52 patients, 20 of whom had tubular dysfunction. The characteristics of this subset of patients were similar to those of the remaining patients (Supplementary table 2). Subjects with tubular dysfunction showed significantly higher KIM-1 urinary levels compared to those without (4.7 [3.4-11.3] vs 2.1 [1.0-3.4] ng/mg, Mann-Whitney test, p<0.001) and a significant correlation between baseline α1-MCR and KIM-1 levels was found (Pearson's r=0.487, p<0.001).

Overall, 22/135 patients (16.3%) exhibited glomerular damage (ACR > 30 mg/g). Of these, 18 (81.8%) showed moderately increased and 4 (18.2%) severely increased ACR values. In 12/22 (54.5%) cases glomerular damage coexisted with tubular dysfunction and these patients showed higher levels of ACR (76.8 [62.5-415.2]) compared to those with glomerular damage alone (40 [35.6-88.0] mg/g, Mann-Whitney test, p=0.03), but similar α1-MCR levels (21 [17.25-29.25])

compared to those with tubular dysfunction alone (22 [16.5-27.5] μg/mg, Mann-Whitney test, p=0.985) .

Cryoglobulinemia was more frequent in subjects with compared to those without glomerular damage, but not significantly (45.4% and 25.7% respectively, p=0.06), and the prevalence of cryoglobulinemia was comparable in patients with or without tubular dysfunction (25% and 31% respectively, p=0.578).

Renal damage evaluation after antiviral treatment

Patients were treated with different DAA regimens (Supplementary table 3), 75.6% containing ribavirin and 80.7% sofosbuvir. The flow chart of the study patients is reported in Figure 1. SVR was achieved by 95.6% (129/135) of patients. The reasons of treatment failure were relapse (2 patients: one HCV genotype 1b infected woman treated with sofosbuvir, simeprevir and ribavirin for 12 weeks; one HCV genotype 3 infected man affected by diabetes and obesity and treated with sofosbuvir, daclatasvir and ribavirin for 24 weeks), death (2 patients: one acute liver failure during therapy, one acute cholecystitis and sepsis 4 weeks after end of treatment) and treatment discontinuation (2 patients). Five months after the end of treatment one patient died due to a complicated hip fracture. Thirteen patients were lost to follow-up after SVR, therefore 115 patients were assessed at FU-6 (Figure 1).

Diabetes remained controlled in all the 26 diabetic subjects enrolled in the study. In fact, Haemoglobin A1c was stably lower than 7% at T0, EOT and FU-6, and anti-diabetic treatment did not require modifications. In patients with arterial hypertension blood pressure remained controlled and anti-hypertensive treatment remained unchanged during the study period.

At FU-6 a slight decrease of e-GFR was observed from 89.4 ± 16.9 mL/min/1.73 m² to 84.9 ± 17.5 mL/min/1.73 m² (paired-sample t-test, $p<0.001$), corresponding to a 5% percentage reduction.

Twenty-eight patients with baseline tubular dysfunction were examined after antiviral treatment. A significant reduction of α1-MCR levels from 21.0 (18.0-30.2) μg/mg to 10.5 (7.2-21.2) μg/mg (Wilcoxon test, p=0.009) and of KIM-1 levels from 4.7 (3.4-11.3) ng/mg to 3.5 (1.8-7.2) ng/mg (Wilcoxon test, p=0.04) were observed. Tubular dysfunction recovered in 16 patients (57.1%). Baseline e-GFR values of these patients were higher (91.5 mL/min/1.73m^2) compared to those who did not recover (75.0 mL/min/1.73m^2) (Mann-Whitney test, p=0.029) (Table 3). The resolution of tubular dysfunction was unrelated with diabetes, arterial hypertension or their combination (Supplementary Table 1).

Surprisingly, *de novo* proximal tubular dysfunction at FU6 occurred in 11/87 (12.6%) patients who showed a significant increase of α1-MCR levels from 4.0 [0-11] μg/mg to 22.0 [15-33] μg/mg (Wilcoxon test, p=0.003). KIM-1 levels, available in 8/11 patients (72.7%), resulted persistently increased in 6 (Supplementary figure 1). Patients who developed tubular dysfunction were older (74 [64-79] vs 59 [52-69] years, p=0.009) and were more frequently female (63.6% vs 30.3%, p=0.03) compared to those who did not develop it (Supplementary table 4). In multiple logistic regression analysis only female gender predicted significantly de novo proximal tubular dysfunction (OR=4.81, 95% CI 1.06-21.8, p<0.05). Notably, all patients with *de novo* tubular dysfunction had been treated with an antiviral regimen including sofosbuvir, either alone (3/14, 21.4%) or in combination (8/57, 14.0%). The logistic regression model to predict de novo tubular dysfunction, using as predictors each drug and their combination, age and gender, failed to converge, probably due to the small number of events.

After antiviral treatment, glomerular damage resolved in 15/22 (68.2%) patients and resolving patients exhibited significantly lower baseline ACR values compared to non-resolving ones (53.8 [38-75] mg/g versus 240 [78.6-609] mg/g, p=0.002) (Table 4). ROC curve analysis showed that ACR values lower than 77 mg/g predicted resolution of glomerular damage after successful HCV treatment (AUC=0.895, sensitivity 85%. specificity 80%) (Figure 2).

Interestingly, all patients who did not resolve glomerular damage had a concomitant tubular dysfunction and their baseline α1-MCR values were significantly higher (21 [20-31] vs 7 [3.5-14.5] μg/mg, p=0.003) compared to patients who resolved it (Table 4). Moreover, all patients without diabetes, but two, resolved the glomerular damage. Of the two non-diabetic subjects who did not resolve, one developed an acute kidney injury and heart failure while on antiviral treatment, the other developed multiple myeloma during follow up. Notably glomerular damage recovered also in 5 out of 10 diabetic patients.

DISCUSSION

In this report, we provide evidence that tubular dysfunction is an unrecognized feature of HCV-related kidney disease in Child-Pugh A cirrhotic patients with a normal glomerular filtration rate. Recently, tubular dysfunction has acquired an increasingly important pathogenetic role in the development of CKD and some authors suggest that tubular damage is a better predictor of renal

function decrease over time than glomerular injury (22, 41), therefore the present finding may shed new light on the mechanisms of renal damage in HCV cirrhotic patients.

Although it is well known that HCV patients are at increased risk of developing CKD (42-45), until now much more attention has been given to the glomerular side of the HCV related kidney disease (11-15). Our study confirms that glomerular damage is a uniform component of HCV related renal disease. In fact, the proportion of patients with moderate albuminuria (13.3%) was similar to the proportion (12.4%) reported among 362 HCV subjects in the study of Liangpunsakul et al. (46), indicating the consistency of the finding in different populations.

On the other side, the potential pathogenetic role of HCV infection in inducing tubulointerstitial injury is supported by the earlier detection of HCV core protein and HCV-RNA replicative strand in tubular epithelial cells from HCV patients with or without glomerular disease (16-18) and by its association with tubulointerstitial inflammatory cell infiltration and fibrosis (18). However, until now, no clinical study evaluated *in vivo* the integrity of tubular function in HCV infected patients.

Though none of the aforementioned studies specified in which portion of the tubule HCV was detected, on the diagnostic perspective we selected two biomarkers of proximal tubular dysfunction because other viruses, such as Zika virus - another member of the *Flaviviridae* family - are able to selectively infect human renal proximal tubular epithelial cells (hRPTEpiCs) (47). Therefore, we employed urinary α1-MCR and KIM-1 because they have proved to be accurate biomarkers for early detection of proximal tubular dysfunction/damage (23, 24, 25, 48, 49). In this way, we demonstrated that α1-MCR values significantly correlated with KIM-1 levels ($r=0.487$, $p<0.001$) and tubular dysfunction was a part of the HCV kidney disease observed in cirrhotic patients. Another study, that examined HCV-HIV coinfected patients, found that HCV infection was associated with 22% higher α1-MCR levels (95% CI 2% to 46%; $p=0.03$) (49), indicating that also in the context of coinfection, HCV carries an excess risk of tubular dysfunction.

In multiple logistic regression analysis, glomerular damage and the concomitant presence of diabetes and hypertension were the only predictors significantly associated with tubular dysfunction, despite normal e-GFR. Therefore, in cirrhotic HCV patients, particularly in those with glomerular damage (ACR > 30 mg/g) and comorbidities, the assessment of markers of tubular dysfunction, such as α1-MCR levels, may be suggested even in subjects with normal or nearly normal e-GFR values.

Novel DAAs have revolutionized HCV treatment allowing high rates of SVR with an excellent safety profile (26). However, a slight and sometimes transient decrease of e-GFR has been described during and after DAAs treatment (50, 51). We have observed a mean reduction of e-GFR of 4.9 mL/min/1.73 m² in the 6 months after DAAs treatment that, though statistically significant, was not clinically meaningful. The same result has been recently reported in a real life cohort of 931 patients after a 12 weeks treatment with DAAs (51). Moreover, treatment with sofosbuvir was associated with a reduction of eGFR in CKD stage 1 patients (49) and sustained eGFR decrease 12 weeks after treatment in two more series of patients. (50). Longer follow-up is needed to explore the time trend of e-GFR in patients treated with DAAs, but the potential benefits of DAA treatment outweigh potential harms for patients with CKD stages 1-3b, independently of the choice of DAA (51).

We report that, in HCV cirrhotic patients, the prevalence of tubular dysfunction was higher than glomerular damage (23.7% and 16.3%, respectively) and viral clearance induced a significant reduction of α1-MCR and KIM-1 levels from baseline to FU-6 with normalization of α1-MCR values in 57.1% of patients. In addition, patients who did not resolve tubular dysfunction had significantly lower e-GFR values (75 mL/min/1.73m^2) compared to those who resolved it (91.5 mL/min/1.73m^2), independently of age. The resolution of tubular dysfunction was independent of the presence of comorbidities, such as diabetes, arterial hypertension, or both. Therefore, we speculate that the tubular dysfunction detected before HCV treatment may resolve in a proportion of patients following HCV cure, suggesting a possible relationship between the infection and the tubular injury. However, once the filtration rate is reduced, although within the nearly normal ranges, viral clearance is not sufficient for the resolution of the tubular dysfunction.

Interestingly, the resolution of glomerular damage was more probable in the absence of tubular dysfunction. These results are in line with the accepted central role of tubular damage in the prognosis of glomerular damage (22) and are in agreement with Kasuno and colleagues who suggested that tubular damage was not secondary to advanced glomerulopathy but was induced by the virus itself (18).

Finally, despite viral clearance, 12.6% of patients, with controlled diabetes and hypertension, exhibited a *de-novo* proximal tubular dysfunction, that was predicted by female gender. This event~~, which occurred only in patients treated with sofosbuvir,~~ might be due to a toxic effect of the drugs. In the present study de novo tubular dysfunction tended to occur more frequently in subjects treated with sofosbuvir (either alone or in combination with ribavirin) compared to those

treated with other DAAs. Although sofosbuvir is mainly eliminated by kidneys (55, 56), its impact on tubular function has been poorly studied and only one published retrospective cohort study demonstrated a significant increase of mean neutrophil gelatinase-associated lipocalin (NGAL) levels, another marker of tubular kidney injury, from baseline to 12 weeks after the end of therapy in 18 patients who received sofosbuvir based DAA regimens (57). The renal excretion of ribavirin accounts for 40% of its clearance, but its renal toxicity has been rarely documented in the association with interferon or DAA treatment for HCV infection (58). The present study supports this hypothesis, since the appearance of the novo tubular dysfunction was similar in patients treated with or without ribavirin. Moreover, the logistic regression model to predict the appearance of de novo tubular dysfunction according to the drug used (sofosbuvir, ribavirin or the combination) failed to converge due to the small number of events. Thus, the long-term effect of sofosbuvir on tubular function remains actually unclear and should be further investigated.

Our study has some limitations. First, the small number of patients with each type of renal damage limits our ability to conduct subgroup analyses. Second, the diagnosis of renal damage (glomerular and tubular) has been performed by reliable markers, but not by kidney biopsy. Third, although α1-MCR is a commonly accepted indicator of tubular dysfunction, it has been mainly applied to HIV infected and diabetic subjects (23, 24). Fourth, longer follow-up is needed to examine the evolution of persistent tubular dysfunction after viral clearance and its impact on kidney function. Fifth, the assessment of urinary KIM-1 levels has been performed only in a subset of patients, limiting the strength of the results, although the characteristics of this subset of patients were similar to those of the remaining patients. Sixth, the cirrhotic condition may be a confounding variable in the interpretation of the pathophysiology of renal damage, but we had the possibility to enroll only patients with cirrhosis on the basis of eligibility criteria for receiving DAAS in 2015, as described. We excluded patients with a more compromised situation to reduce additional confounding factors, moreover we evaluated the absence of signs suggesting renal involvement secondary to the cirrhosis itself, such as heavy proteinuria, microhaematuria, acute on chronic liver failure and/or acute deterioration of renal function.

In conclusion, tubular dysfunction is an unrecognized feature of HCV related kidney disease in compensated cirrhotic patients with a normal or mildly decreased glomerular filtration rate. Therefore an optimal approach for a complete and early recognition of renal disease should include, in addition to e-GFR and ACR, the assessment of markers of tubular dysfunction, such as α1-MCR levels, particularly in HCV cirrhotic subjects with glomerular damage, diabetes and

hypertension. Even if tubular dysfunction resolves in the majority of patients after viral clearance, the persistence or *de novo* developing of tubular dysfunction after DAA treatment is noteworthy and its long-term impact on kidney function is presently unknown. Therefore, given the central role of the tubule in CKD progression, a careful evaluation of tubular dysfunction should be performed in cirrhotic subjects after viral clearance to monitor kidney function over time and to plan a secondary prevention.

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FIGURES

Figure 1: Flow chart of study patients

Figure 2: ROC analysis: ACR predicting glomerular damage resolution

Supplementary Figure 1: Individual patient data on KIM-1 levels at T0 and FU-6 in subjects with *de novo* proximal tubular dysfunction

Lay summary

Hepatitis C virus (HCV) infection is a systemic disease, associated with several extra-hepatic manifestations. The kidney is one of the main targets, usually demonstrating an involvement on the glomerular side.

With this paper we evidence the involvement of tubular kidney function in HCV compensated cirrhotic patients with normal kidney function, underlying the importance of a complete and early evaluation of renal disease that explores this aspect.

The treatment of HCV infection by direct acting antiviral agents (DAAs) suggest a potential beneficial effect on tubular kidney dysfunction, but further studies are needed to confirm this hypothesis.

Table 1: Characteristics of enrolled patients (N=135)

Age, yr	62.6 ± 10.8
Male gender	83 (61.5%)
Body-mass index, kg/m^2	24.9 ± 3.4
Only diabetes	26 (19.3%)
Only arterial hypertension	56 (41.5%)
Combined diabetes and arterial hypertension	16 (11.9%)
Cryoglobulinemia	39 (28.9%)
eGFR, ml/min/1.73m^2	89.7 ± 16.1
KDIGO GFR categories	
G1 (>90 ml/min)	75 (55.5%)
G2 (60-89 ml/min)	51 (37.8%)
G3a (45-59 ml/min)	9 (6.7%)
Liver stiffness, KPa	23.8 ± 13.9
HCV-RNA, Log$_{10}$ UI/ml	5.8 ± 0.9
HCV genotype	
1a	26 (19.2%)
1b	63 (46.7%)
2	18 (13.3 %)
3	19 (14.1%)
4	9 (6.7%)
Previous antiviral treatment	
Naïve	51 (37.8%)
Null responder	45 (33.3%)
Partial responder	2 (1.5%)
Relapse	20 (14.8%)
Virologic breakthrough	3 (2.2%)
Discontinued	14 (10.4%)

Abbreviations: eGFR - estimated Glomerular Filtration Rate; HCV - hepatitis C virus; KDIGO - Kidney Disease Improving Global Outcome

Table 2: Comparison of HCV patients with and without proximal tubular dysfunction

	No proximal tubular dysfunction 103/135 (76.3%)	**Proximal tubular dysfunction** 32/135 (23.7%)	**P**
Age, yr	61.0 (53.0-69.0)	66.0 (59.2-75.7)	0.020
Male gender	65/103 (63.1%)	18/32 (56.2%)	0.485
Only diabetes	14/103 (13.6%)	12/32 (37.5%)	0.003
Only arterial hypertension	36/103 (34.9%)	20/32 (62.5%)	0.006
Combined diabetes and arterial hypertension	8/103 (7.8%)	8/32 (25%)	0.008
BMI, Kg/m^2	25.0 (22.0-27.0)	26.0 (23.0-27.0)	0.205
Liver stiffness, Kpa	18.0 (14.0-27.0)	21.5 (14.7-30.7)	0.471
Platelet x10^6/mm^3	111.0 (76.0-159.0)	138.5 (79.0-164.7)	0.464
ALT, U/L	76.5 (51.5-118.0)	54.2 (74.5-112.2)	0.888
eGFR, ml/min/1.73m^2	93.0 (84.0-102.0)	84.5 (74.5-97.2)	0.044
Glomerular damage (ACR ≥ 30 mg/g)	10/103 (9.7%)	12/32 (37.5%)	<0.001
HCV-RNA, Log$_{10}$UI/ml	6.0 (5.4-6.5)	6.1 (5.0-6.4)	0.453

Abbreviations: ALT - alanine aminotransferase; BMI - Body Mass Index; eGFR - estimated Glomerular Filtration Rate; HCV - hepatitis C virus.

Table 3: Comparison of HCV patients with and without proximal tubular dysfunction resolution

	Proximal tubular dysfunction resolution 16/28 (57.1%)	**No proximal tubular dysfnction resolution 12/28 (42.9%)**	**P**
Age, yr	60.5 (59-74)	70.5 (62.7-78)	0.146
Male gender	10/16 (62.5%)	6/12 (50.0%)	0.508
Diabetes	6/16 (37.5%)	6/12 (50.0%)	0.508
Arterial Hypertension	9/16 (56.2%)	9/12 (75.0%)	0.306
BMI, kg/m^2	26 (23.5-27)	25.5 (23-27.7)	0.837
Liver stiffness, KPa	20.5 (14.7-26)	22.5 (16.7-40.5)	0.484
Platelet x 10^6/mm^3	150.5 (121-176.2)	99.5 (64.2-163.7)	0.371
ALT, U/L	78 (67-166)	72 (37-106.5)	0.371
e-GFR, ml/min/1.73m^2	91.5 (83.2-102.2)	75 (57.5-92)	0.029
ACR, mg/g	13.3 (0.8-46.5)	43.9 (2-198.7)	0.371
Baseline glomerular damage	4/16 (25.0%)	6/12 (50.0%)	0.242
α1-MCR, µg/mg	21.5 (18.5-30.2)	20.0 (17.2-33.7)	0.732
Treatment including Ribavirin	10/16 (62.5%)	6/12 (50.0%)	0.508
Treatment including Sofosbuvir	11/16 (68.7%)	9/12(75%)	0.717

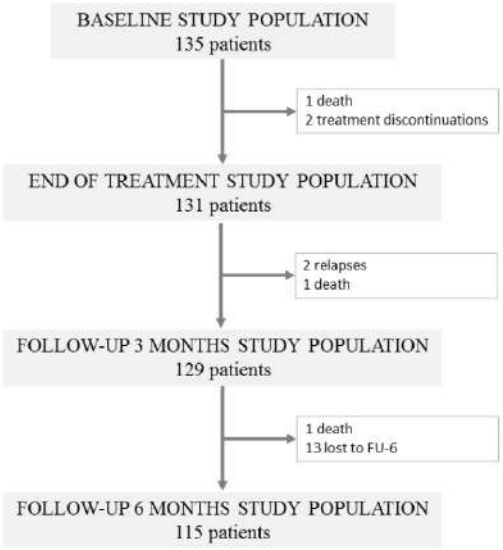
Abbreviations: α1-MCR - α1 microglobulin to creatinine ratio; ACR - urinary albumin to creatinine ratio; ALT - alanine aminotransferase; BMI - Body Mass Index; eGFR - estimated Glomerular Filtration Rate; HCV - hepatitis C virus.

Table 4: Comparison of HCV patients with and without glomerular damage resolution

	Glomerular	**No glomerular**	**P**

	damage resolution 15/22 (68.2%)	**damage resolution 7/22 (31.8%)**	
Age, yr	67 (64-75)	66 (59-69)	0.447
Male gender	8/15 (53.3%)	4/7 (57.1%)	0.348
Diabetes	5/15 (33.3%)	5/7 (71.4%)	0.097
Arterial hypertension	10/15 (66.6%)	5/7 (71.4%)	0.370
BMI, kg/m^2	24 (21-26)	26 (23-28)	0.368
Liver stiffness, KPa	16 (11.7-27.2)	21 (18.5-27)	0.312
Platelet x 10^6/mm^3	125 (79-157)	151 (63-166)	0.837
ALT, U/L	107 (66.5-144.2)	68 (67–77)	0.067
e-GFR, ml/min/1.73m^2	85 (70-92)	83 (73-95)	0.837
ACR, mg/g	53.8 (38 – 75)	240 (78.6-609)	0.002
Baseline proximal tubular dysfunction	5/15 (33.3%)	7/7 (100%)	0.005
α1-MCR, μg/mg	7 (3.5-14.5)	21 (20-31)	0.003
Treatment including Ribavirin	9/15 (60.0%)	5/7 (71.4%)	0.329
Treatment including Sofosbuvir	9/15 (60.0%)	5/7(71.4%)	0.329

Abbreviations: α1-MCR - α1 microglobulin to creatinine ratio; ACR - urinary albumin to creatinine ratio; ALT - alanine aminotransferase; BMI - Body Mass Index; eGFR - estimated Glomerular Filtration Rate; HCV - hepatitis C virus.



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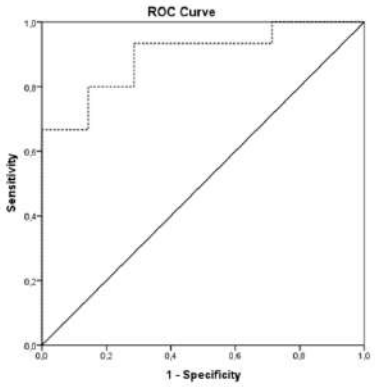

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