Low Complement C4 Predicts Improvement of Kidney Function After Direct-Acting Antiviral Therapy for Hepatitis C Virus

Meghan E. Sise,[1]* Ian Strohbehn [iD] ,[2]* Donald Chute,[2] Kathleen E. Corey,[2] Dahlene N. Fusco,[3] Venkata S Sabbisetti,[4] Sushrut S. Waikar,[4,5] and Raymond T. Chung[2]

Direct-acting antiviral therapies (DAAs) may improve kidney function and proteinuria in certain patients with hepatitis C infection (HCV) and chronic kidney disease (CKD). To improve our understanding of HCV-mediated kidney dysfunction, we aimed to evaluate the baseline predictors of improvement in proteinuria after DAAs in a single-arm, pilot, clinical trial of ledipasvir 90 mg/sofosbuvir 400 mg once daily for patients with HCV genotype 1 or 4 infection and proteinuric CKD (≥300 mg proteinuria per gram creatinine). Plasma biomarkers of complement system (C3 and C4) and urinary kidney injury biomarkers were measured at baseline, 8 weeks on treatment, 12 weeks following treatment, and 1 year following treatment. We then conducted a retrospective cohort study of patients at Partners Healthcare who had baseline complement component 4 (C4) measured before DAAs for HCV and evaluated the change in estimated glomerular filtration rate (eGFR) before and after therapy. Ten patients with HCV and proteinuric CKD were enrolled in the trial. The mean age was 64 years, 70% male, 70% white, and 30% black. Baseline creatinine was 1.25 mg/dL (SD 0.44), eGFR was 65 mL/min/1.73 m^2 (SD 29), and proteinuria was 0.98 g/g creatinine (SD 0.7). Sustained virologic response at 12 weeks was achieved by 80% of patients. Patients with low baseline C4 had improved proteinuria, urinary neutrophil gelatinase-associated lipocalin, and interleukin-18 after ledipasvir and sofosbuvir treatment. The retrospective study included 50 patients with CKD and HCV. Twenty patients (40%) had low baseline C4; these patients significantly improved their eGFR (+3.4 ± 11.2 mL/min/1.73 m^2) compared to those with normal baseline C4 (−4.4 ± 12.2 mL/min/1.73 m^2; P = 0.028). *Conclusion:* Low C4 may be a marker of kidney dysfunction that improves with DAA therapy. (*Hepatology Communications* 2020;4:1206-1217).

Epidemiologic studies show that hepatitis C virus (HCV) infection increases the risk of incident chronic kidney disease (CKD) and accelerates the progression of CKD to end-stage renal disease (ESRD).[(1-6)] However, the mechanisms that drive CKD progression are not well understood.

Chronic HCV may lead to cryoglobulinemic glomerulonephritis, resulting from immune complex deposition, yet there are other potential mechanisms that may accelerate CKD progression in patients with HCV infection.

With the advent of new, well-tolerated direct-acting antiviral therapies (DAAs), it is now possible to treat all genotypes of HCV infection in patients with CKD and ESRD.[7-9] We have recently shown that DAA therapy may improve kidney function (estimated glomerular filtration rate [eGFR] and proteinuria) in patients with CKD.[10-12] However, the mechanism by which DAAs improve kidney disease and proteinuria is unclear. The aim of this study was to characterize changes in kidney function during DAA therapy, and to determine whether any baseline characteristics could predict which patients had improvement in kidney function markers during DAA therapy.

Methods

From July 2015 to May 2018 we conducted a prospective, single-arm pilot trial to assess the effect of co-formulated ledipasvir 90 mg and sofosbuvir 400 mg (LDV/SOF) on kidney function in patients with proteinuric CKD. This investigator-initiated trial was funded by Gilead Sciences and the protocol was approved by the institutional review board at Partners Healthcare. Eligible patients were at least 18 years old, infected with HCV genotype 1 or 4, and diagnosed with proteinuric CKD (defined by at least 300 mg/g protein to creatinine ratio on two freshly voided urine samples). Full inclusion and exclusion criteria are given in Supporting Table S1.

Patients underwent two screening visits up to 60 days before the first dose of LDV/SOF. Medical history and concomitant medications were reviewed. Blood and urine tests, physical exam, and 12-lead electrocardiograms were performed. Additionally, a FibroScan was done to evaluate for cirrhosis (if a liver biopsy was not available); score of greater than 12.5 kPa was considered diagnostic of cirrhosis. On-therapy follow-up visits occurred at 2 weeks, 4 weeks, 8 weeks, and 12 weeks (end of treatment). Patients returned at 4 weeks, 12 weeks, and 24 weeks after therapy and completed the study at 1 year. Sustained virologic response (SVR) was defined as a negative HCV RNA test 12 weeks after completion of LDF/SOF (Fig. 1). At each follow-up visit, the following items were performed: physical exam, vital signs, weight, review of adverse events related to study procedures and concomitant medications, and blood and urine sample collection. Fasting blood samples were taken at baseline, 8 weeks on treatment, 12 weeks following treatment, and 1 year following treatment.

The primary endpoint assessed was the percent change in proteinuria. Because of the inherent variability in spot proteinuria, the *baseline proteinuria* was determined by averaging the value from the screening and start of treatment visits. *On-treatment proteinuria* was determined by the value at week 8, and *posttreatment proteinuria* was defined by the average proteinuria at week 12 (PT+12), week 24 (PT+24), and week 40 (PT+40) visits. Improvement in proteinuria was defined by a 25% decline in proteinuria from baseline to post treatment. Worsening of proteinuria was defined by a 25% increase in proteinuria from baseline to post treatment. Stable proteinuria was defined as less than 25% change in either direction from baseline.

ARTICLE INFORMATION:

From the [1]Division of Nephrology, Department of Medicine, Massachusetts General Hospital, Boston, MA; [2]Gastrointestinal Division, Department of Medicine, Massachusetts General Hospital, Boston, MA; [3]Department of Medicine, Infectious Diseases Section, Tulane University School of Medicine, New Orleans, LA; [4]Division of Nephrology, Brigham and Women's Hospital, Boston, MA; [5]Section of Nephrology, Boston Medical Center, Boston, MA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Raymond T. Chung, M.D.
Gastrointestinal Division, Department of Medicine
Massachusetts General Hospital
55 Fruit Street

Boston, MA 02114
E-mail: Chung.Raymond@mgh.harvard.edu
Tel.: 617-724-6006
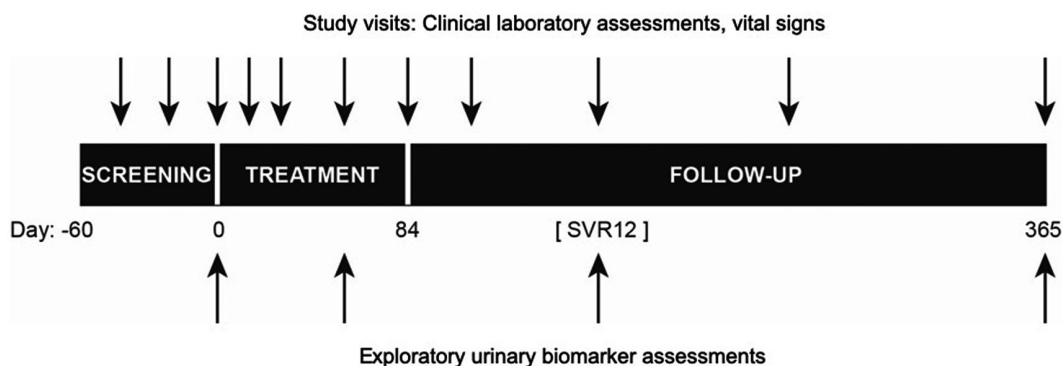
FIG. 1. Pilot trial schematic and timeline for follow-up visits. The arrows above the timeline represent patient visits; clinical laboratory assessments were conducted twice during the screening period: at baseline, week 2, week 4, week 8, and week 12 of treatment, and at 4, 12, 24, and 40 weeks after treatment ended. The arrows below the timeline indicate the timepoint at which cryoglobulin levels, complement levels, and exploratory urinary biomarkers assessments were conducted: baseline, week 8 on treatment, 12 weeks following treatment, and 40 weeks following treatment.

Secondary endpoints included mean changes in serum creatinine and cystatin-C levels from baseline, on treatment, and post treatment. Cryoglobulin, complement levels, C-reactive protein, and urinary kidney injury biomarkers were measured at four timepoints (Fig. 1). Low complement component 4 (C4) was defined as less than 12 mg/dL, per the reference range of our laboratory. Hemoglobin A1c was measured at baseline, SVR12, and SVR40. Urinary biomarkers, including kidney injury marker-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and interleukin-18 (IL-18) were measured using microbead-based assay on a Luminex platform (Austin, TX).[13]

Prompted by results of this pilot study, we also conducted a retrospective cohort analysis of all patients at Partners Healthcare with CKD defined by eGFR < 60 mL/min/1.73 m². The details of the overall cohort of DAA-treated patients have been previously published.[12] DAA therapy was prescribed at the discretion of the treating physician; treatment regimens depended on the genotype, presence of cirrhosis, and prior treatment experience. We obtained patient laboratory data from the Research Patient Data Registry at Partners Healthcare System. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[14] We compared the average eGFR before and after DAAs in all patients who had baseline complement C4 levels measured before initiating DAA therapy.[12] Low C4 was again defined as less than 12 mg/dL, per the reference range of our

laboratory. Baseline characteristics of the cohort were described using mean ± SD, median and interquartile range (IQR), or percent. A univariate linear regression model was used to determine baseline factors associated with change in eGFR improvement after DAA therapy; odds ratios and 95% confidence intervals were used to summarize the results of this model. We used chart review to determine whether patients with low C4 had clinical features of mixed cryoglobulinemic syndrome.

All analyses were performed with Stata Version 13 (StataCorp, College Station, TX). A two-sided *P* value of less than 0.05 was considered to indicate statistical significance. The institutional review board at Partners Healthcare System approved this clinical trial protocol, and all participants underwent full informed consent by a study physician. The trial was registered at Clinicaltrials.gov (NCT02503735). For the retrospective study, the institutional review board waived the need for informed consent.

Results

BASELINE PATIENT DEMOGRAPHICS AND CHARACTERISTICS OF PILOT TRIAL PARTICIPANTS

Fourteen patients signed informed consent for clinical trial participation; 4 were excluded due to

medical or psychiatric comorbidities precluding study participation. The baseline demographics and clinical characteristics of the 10 patients who began LDV/SOF are provided in Table 1. In total, 70% were male. Age ranged from 51 to 82 years (mean = 64). With respect to comorbidities, 40% had compensated cirrhosis, 90% had hypertension, and 50% had diabetes. With respect to treatment experience, 60% were treatment-naïve and 40% had previously failed interferon-based therapy. Most had genotype 1 infection (3 had genotype 1A, 4 had genotype 1B), 2 had genotype 4, and 1 patient had mixed genotype 1/4 infection. Median baseline viral load was 1.5×10^6 IU/mL (IQR 5.6×10^5 to 4.2×10^6 IU/mL).

Three patients had a eGFR of 90 mL/min/1.73 m^2 or higher, 2 had a eGFR of 60-89 mL/min/1.73 m^2,

TABLE 1. BASELINE CHARACTERISTICS OF THE PILOT TRIAL (n = 10) PARTICIPANTS AND SUBJECTS INCLUDED IN THE RETROSPECTIVE COHORT STUDY (n = 50)

	Clinical Trial Subjects (n = 10)	Retrospective Subjects (n = 50)
Age at baseline, mean (SD)	64 (11)	64 (9.6)
Male, n (%)	7 (70)	31 (62)
Race, n (%)		
White, non-Hispanic	7 (70)	24 (48)
Black, non-Hispanic	3 (30)	15 (30)
Asian, non-Hispanic	–	4 (8)
Hispanic	–	7 (14)
HCV genotype, n (%)		
1a	3 (30)	27 (54)
1b	4 (40)	11 (22)
2	–	6 (12)
3	–	1 (2)
4	2 (20)	5 (10)
Mixed	1 (10)	–
eGFR groups (CKD stages), n (%)		
≥90 mL/min/1.73 m^2 (CKD stage 1)	3 (30)	–
60-89 mL/min/1.73 m^2 (CKD stage 2)	2 (20)	–
30-59 mL/min/1.73 m^2 (CKD stage 3)	5 (50)	47 (94)
<30 mL/min/1.73 m^2 (CKD stage 4+)	–	3 (6)
Cirrhosis, n (%)	4 (40)	31 (62)
Diabetes, n (%)	5 (50)	24 (48)
Hypertension, n (%)	9 (90)	44 (88)
HIV, n (%)	–	5 (10)
Baseline HCV RNA (IU/mL), median (IQR)	1,573,500 (564,250-4,162,500)	2,980,000 (1,245,000-6,560,000)
Previous HCV treatment experience, n (%)	4 (40)	21 (42)
Baseline creatinine, mean (SD)	–	1.57 (0.41)
Baseline proteinuria, mean SD)	0.98 (0.7)	–
Hypocomplementemia, n (%)		
Low C4	4 (40)	20 (40)
Previous kidney transplant, n (%)	–	5 (10)
Previous liver transplant, n (%)	–	9 (18)
Outcome, n (%)		
SVR12	8 (80)	42 (89)
Relapse	2 (20)	5 (11)
Unknown	–	3
Discontinued early, n (%)	1 (10)	2 (4)

Note: The retrospective cohort includes all patients at Partners Healthcare with CKD undergoing DAAs for chronic HCV infection who had C4 measured before starting the DAA therapy. Patients had to have at least one serum creatinine measured before and after receiving a DAA therapy to calculate change in eGFR.

and 5 had a eGFR of 30-59 mL/min/1.73 m^2. Mean baseline proteinuria was 980 mg/g creatinine (SD = 700 mg/g creatinine). At baseline, 70% had a circulating cryoglobulin detected at 1% cryocrit or higher (median 2% cryocrit, range 1%-4%), and 40% had a positive rheumatoid factor (>30 IU/mL). No patients had low C3 at baseline, but 4 patients (40%) had low C4. Only 1 patient had clinical features of mixed cryoglobulinemic syndrome (vasculitic rash) at the time of treatment initiation.

TREATMENT EFFECTIVENESS AND SAFETY

The 12-week course of LDV/SOF resulted in SVR12 in 80% of study participants. Two patients experienced virologic relapse; 1 was retreated outside this study with glecaprevir-pibrentasvir for 12 weeks and was cured, while the other declined retreatment. Overall, adverse events were mild, with only fatigue, nausea, and headache experienced by more than 1 participant. Only 1

participant experienced a severe adverse event. Three weeks after completing LDV/SOF, he was hospitalized for small bowel obstruction, which was deemed to be unrelated to study participation. One patient discontinued therapy 6 days early due to rising creatinine at the final treatment visit. This was deemed by the primary investigator to be possibly related to study treatment; however, this patient ultimately achieved improvement in proteinuria and creatinine in the posttreatment follow-up period.

EFFECT OF LDV/SOF ON PROTEINURIA, CREATININE LEVELS, AND IMMUNOLOGIC ABNORMALITIES

Five patients experienced improved proteinuria (>25% decline from baseline), 3 worsened (>25% increase from baseline), and 2 had stable proteinuria with treatment (percent change in proteinuria from baseline is shown in Figs. 2 and 4). The characteristics of the patients whose
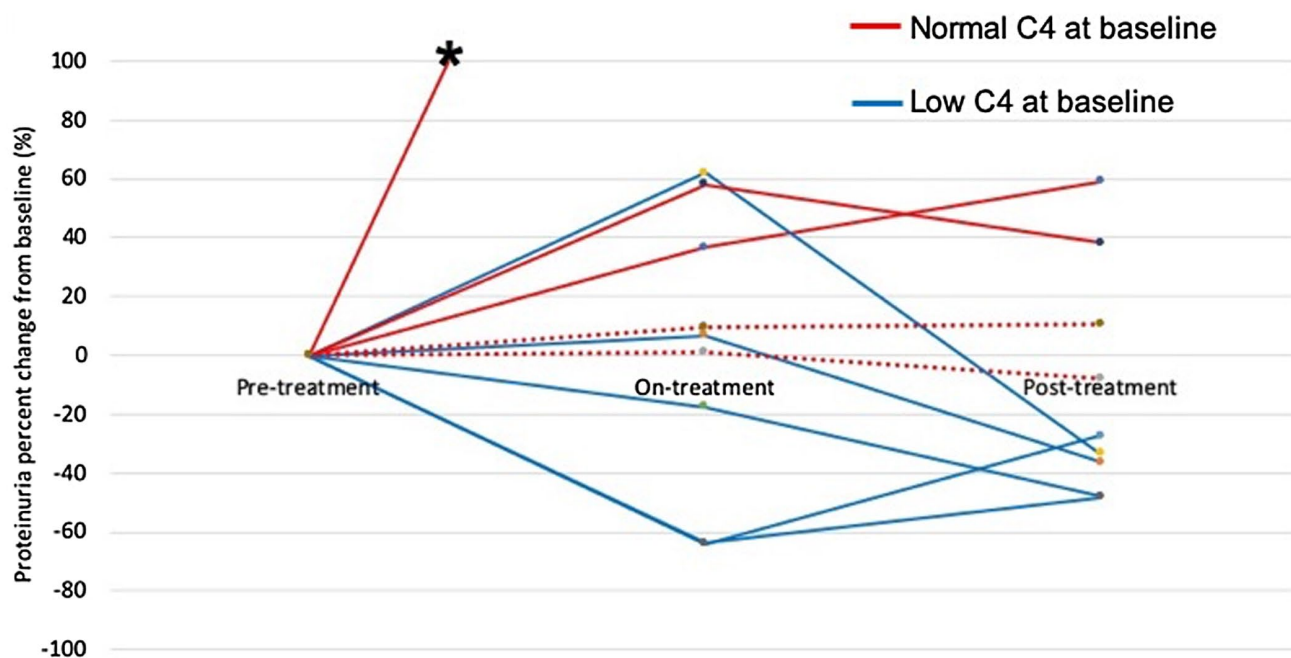
FIG. 2. Proteinuria percent change from baseline by baseline-C4 status. Proteinuria values from pretreatment (average of screening and baseline), on treatment (week 8), and post treatment (average of 12, 24, and 40 weeks following treatment) were compared and presented as percent change from pretreatment. C4 status was determined by the laboratory reference range with a low value designated as <12 mg/dL. Patients with baseline low C4 (blue lines) had, on average, a 36.3% (SD 8.7) decrease in proteinuria from baseline to post treatment, whereas subjects with normal C4 at baseline (red lines) had a 50.6% (SD 105.2) increase in proteinuria from baseline to post treatment (P = 0.1446). Subjects 3 and 10 did not achieve SVR12 and are represented with dotted lines. Represented by the star, subject 8 had a 284% and 252% increase in proteinuria from baseline at the on-treatment timepoint and posttreatment timepoint, respectively.

proteinuria improved compared to those who stayed stable or worsened are presented in Table 2. Visually inspecting the data, we noted that all patients with low C4 (Fig. 2) had improvement in proteinuria with LDF/SOF. Percent change in serum creatinine from baseline

by C4 status is shown in Fig. 3. Again, patients with low C4 at baseline were more likely to have a decline in serum creatinine with SOF/LDV. Raw proteinuria values, serum creatinine, and cystatin C for each timepoint and C4 status are given in Supporting Table S2A-C.

TABLE 2. BASELINE CHARACTERISTICS OF TRIAL PARTICIPANTS WHOSE PROTEINURIA IMPROVED, REMAINED STABLE, OR WORSENED

Proteinuria Status	Baseline Average (g/g)	On Treatment (g/g)	Posttreatment Values (g/g)	Baseline C4 Status	Diabetes	SVR12
Improved	0.46	0.49	0.29	Low	No	Yes
Improved	1.16	1.87	0.77	Low	Yes	Yes
Improved	3.12	1.12	2.26	Low	Yes	Yes
Improved	0.35	0.29	0.18	Normal	No	Yes
Improved	1.72	0.62	0.89	Low	No	Yes
Stable	0.34	0.35	0.32	Normal	No	No
Stable	0.55	0.60	0.60	Normal	No	No
Worsened	0.82	1.12	1.30	Normal	Yes	Yes
Worsened	1.26	1.99	1.74	Normal	Yes	Yes
Worsened	0.79	3.02	2.77	Normal	Yes	Yes

Note: Our primary outcome was the change in proteinuria, defined by urine protein-to-creatinine ratio measured in grams per gram, from baseline average to posttreatment average. Patients who "improved" had at least a 25% reduction in proteinuria. Patients who worsened had at least a 25% increase in proteinuria. Patients who remained stable remained within a 25% margin of their baseline.
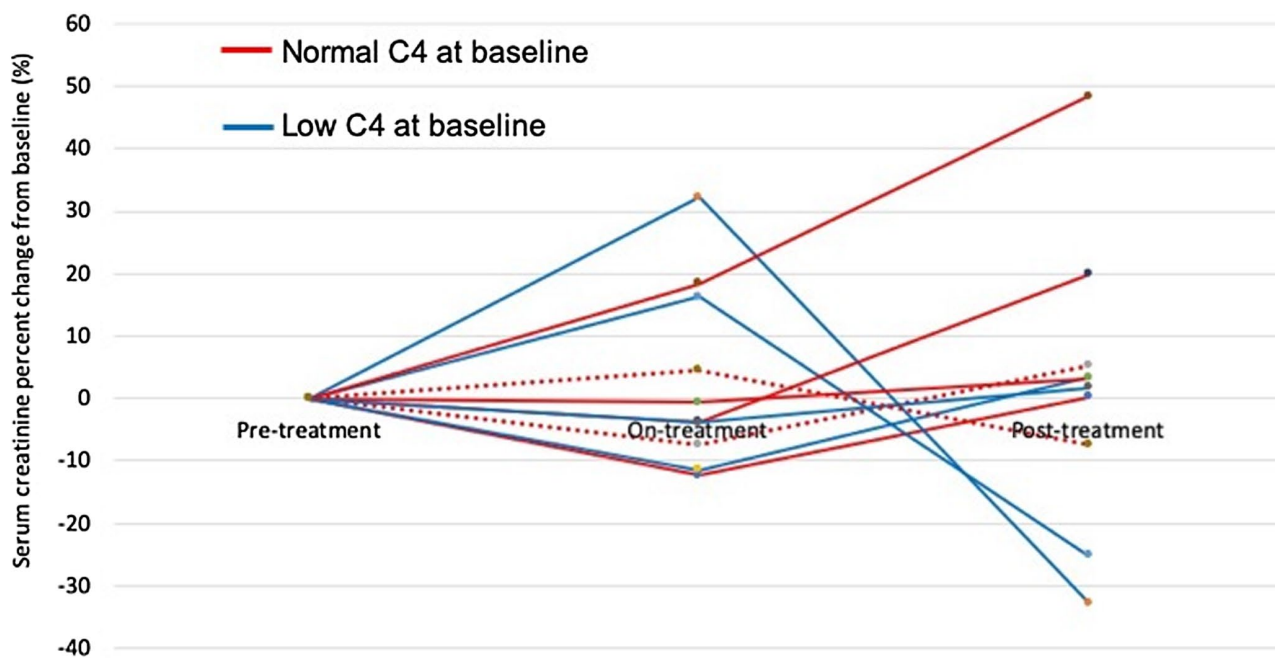
FIG. 3. Serum creatinine percent change from baseline by baseline-C4 status. Serum creatinine values from pretreatment (average of screening and baseline), on treatment (week 8), and post treatment (average of 12, 24, and 40 weeks following treatment) were compared and presented as percent change from pretreatment. C4 status was determined by the laboratory reference range with a low value designated as <12 mg/dL. Subjects 3 and 10 did not achieve SVR12 and are represented with dotted lines.

Most patients with diabetes (3 of 5) had worsening proteinuria (Fig. 4). However, it is important to note that 2 of the diabetic patients had low C4 at baseline, and these patients experienced improvement in proteinuria with LDV/SOF. Of note, glycemic control was very good at baseline in all patients. Glycemic control remained stable throughout the study with mean baseline hemoglobin A1c of 5.9% (SD 0.85), and posttreatment (averaged from SVR12 and SVR40 timepoint) was 6.0% (SD 0.89). No patient had a hemoglobin A1c greater than 7.5 at any point during the study. Of the 3 patients whose proteinuria worsened, each had normal baseline complement C4 levels (Table 2). One patient had a greater than 200% increase in proteinuria from baseline to on-treatment and posttreatment timepoints (Figs. 2 and 4). Despite having controlled blood pressure at study enrollment, this patient had difficulty in controlling hypertension (systolic blood pressures ranging from 150-190 mm Hg) during and after study treatment visits, despite close follow-up with her nephrologist and primary care doctor. Her hemoglobin A1c ranged from 7.%5-8% during the 1-year study period. Percent change in serum creatinine from baseline by diabetes status is shown in Fig. 5, with most patients experiencing stable to worsened creatinine. The lone diabetic patient with improvement in serum creatinine also had low C4. Raw proteinuria values, serum creatinine, and cystatin C for each timepoint and diabetes status are also shown in Supporting Table S2A-C.

After LDV/SOF, cryoglobulin levels disappeared in all but 1 patient (Fig. 6). This individual had persistently low C4 and elevated rheumatoid factor; however, symptoms of mixed cryoglobulinemic syndrome (rash and proteinuria) improved. The remainder of patients had normalization of C4 and decline in rheumatoid factor after LDV/SOF (Supporting Fig. S1A,B).

EXPLORATORY URINARY BIOMARKERS

We stratified urinary biomarker changes by C4 status and found that patients with low C4 at baseline
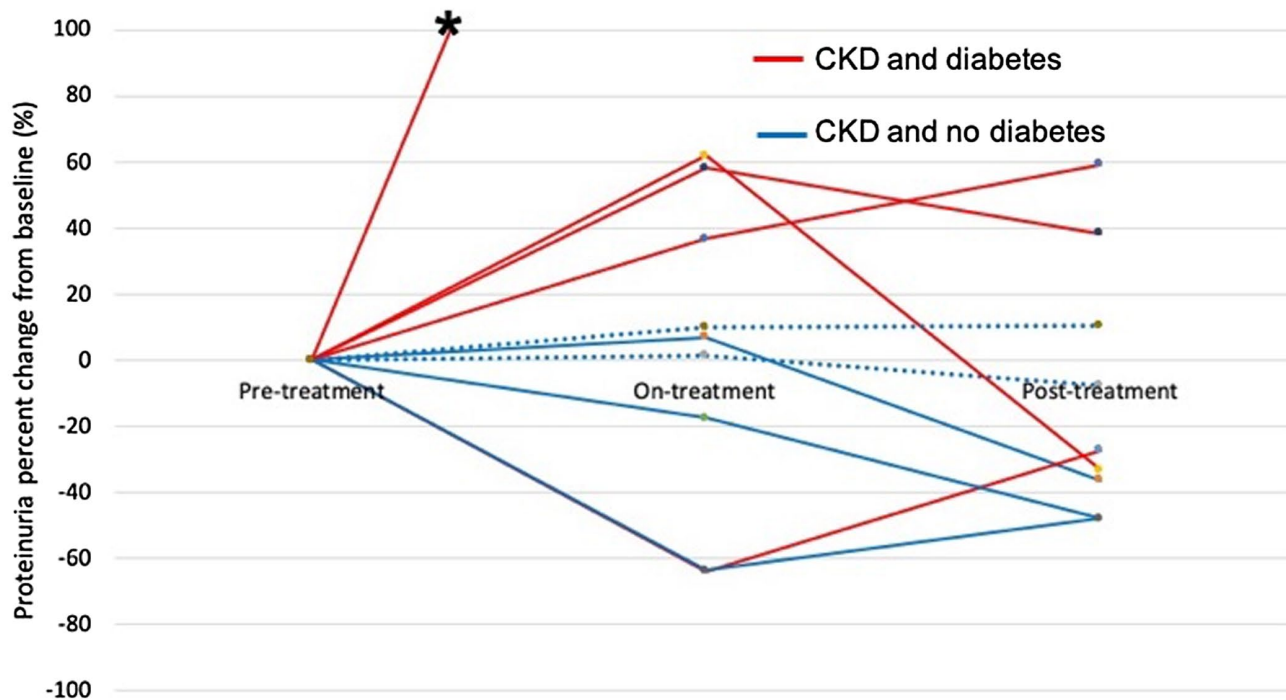
FIG. 4. Proteinuria percent change from baseline by diabetes status. Proteinuria values from pretreatment (average of screening and baseline), on treatment (week 8), and post treatment (average of 12, 24, and 40 weeks following treatment) were compared and presented as percent change from pretreatment. Patients with diabetes (red lines) had, on average, a 57.7% (SD 115.6) increase in proteinuria from baseline to post treatment, whereas subjects without diabetes (blue lines) had a 26.0% (SD 26.2) decrease in proteinuria from baseline to post treatment (P = 0.1063). Subjects 3 and 10 did not achieve SVR12 and are represented by dotted lines. Represented by the star, subject 8 had a 284% and 252% increase in proteinuria from baseline at the on-treatment timepoint and post-treatment timepoint, respectively.
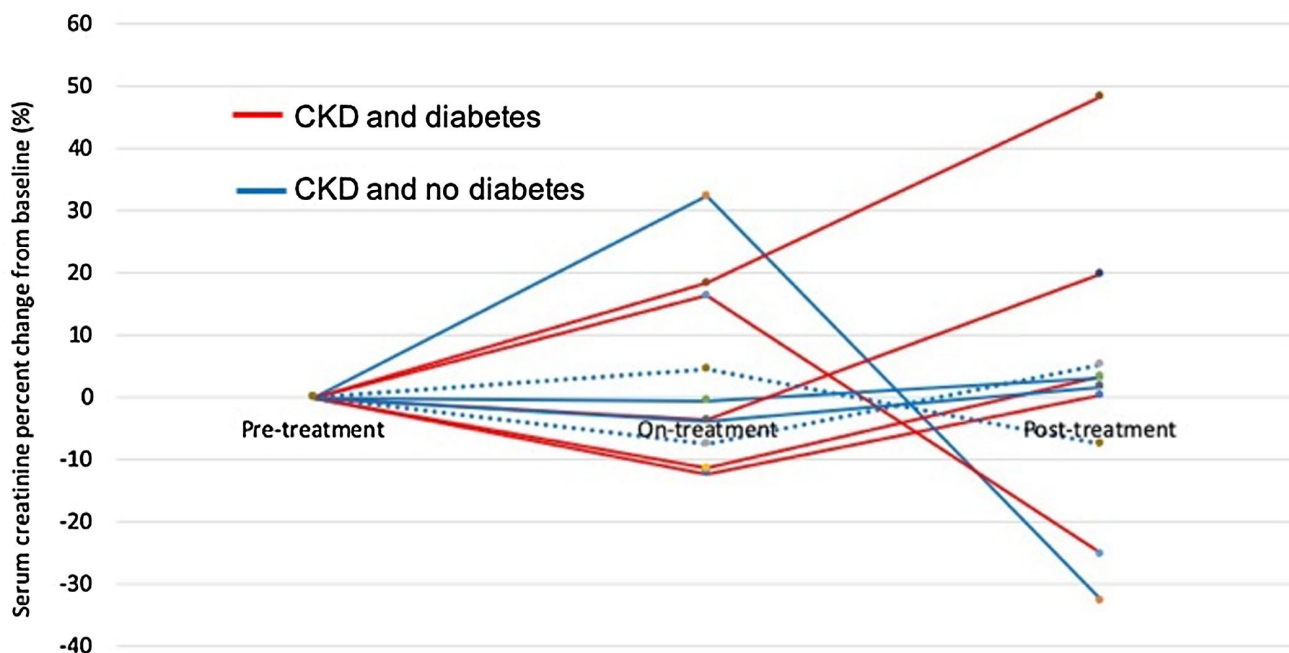
FIG. 5. Serum creatinine percent change from baseline by diabetes status. Serum creatinine values from pretreatment (average of screening and baseline), on treatment (week 8), and post treatment (average of 12, 24, and 40 weeks following treatment) were compared and presented as percent change from pretreatment. Subjects 3 and 10 did not achieve SVR12 and are represented by dotted lines.
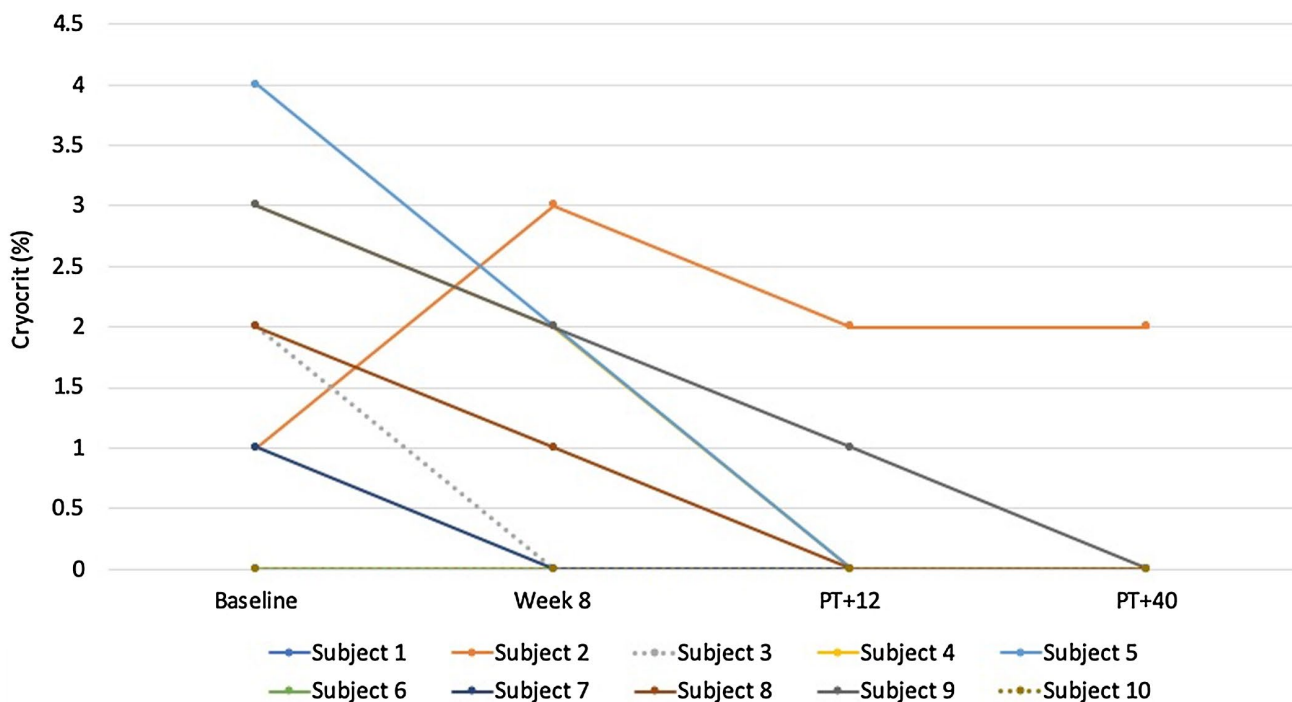
FIG. 6. Cryocrit represented in all patients over the course of treatment. Subject 3 had missing data at PT+12 and PT+40. Subject 5 had missing data at week 8. The trend of subject 4 is obscured by other subject trends: It 3% at baseline, 2% at week 8, 0% at PT+12, and 0% at PT+40. Subjects 3 and 10 did not achieve SVR12 and are represented by dotted lines. Patient identification numbers are consistent in the figures and Supporting Tables throughout.

experienced improving levels of urinary IL-18 and NGAL after starting treatment, while KIM-1 levels remained stable. Urinary biomarkers were stable in patients with normal C4 at baseline, although there was a substantial amount of variability and the margins of error were large (Figs. 7-9). Raw changes in urinary biomarkers are found in Supporting Table S3A-C.

## RETROSPECTIVE COHORT STUDY: PATIENT CHARACTERISTICS

We identified 125 patients with CKD who received DAAs during the study period.[12] Sixty patients with CKD had complement C4 level measured before beginning DAA therapy. Ten were excluded because they lacked follow-up serum creatinine
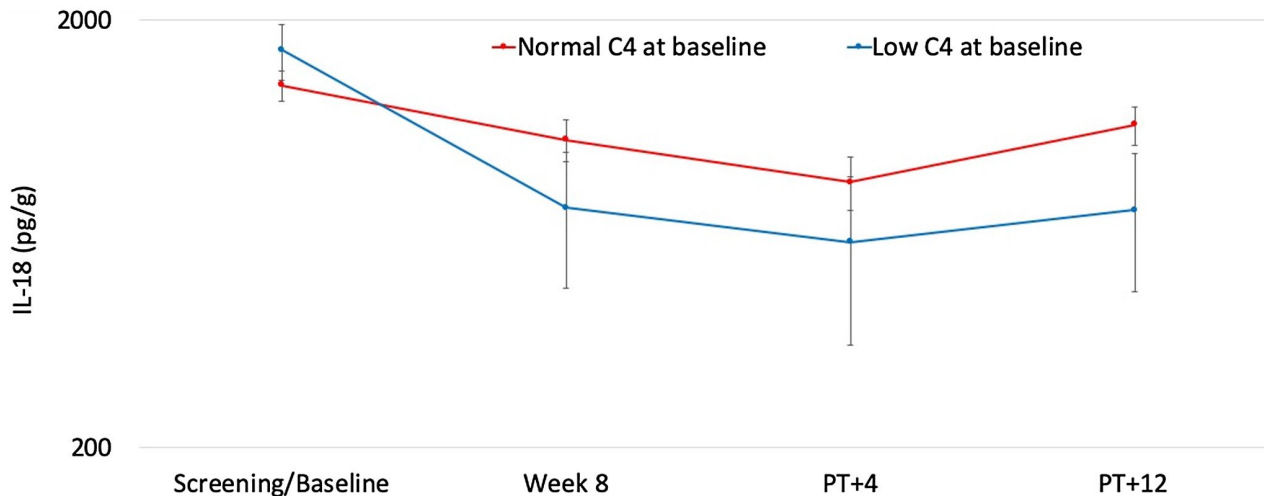
FIG. 7. Average urinary IL-18 values over the study period, by C4 status. Patients were stratified into two groups: low C4 at baseline and normal C4 at baseline. Mean IL-18 and standard error bars are shown at four timepoints throughout the course of treatment, stratified by baseline C4 level. The y-axis is on a base-10 logarithmic scale in response to skewness toward large values.
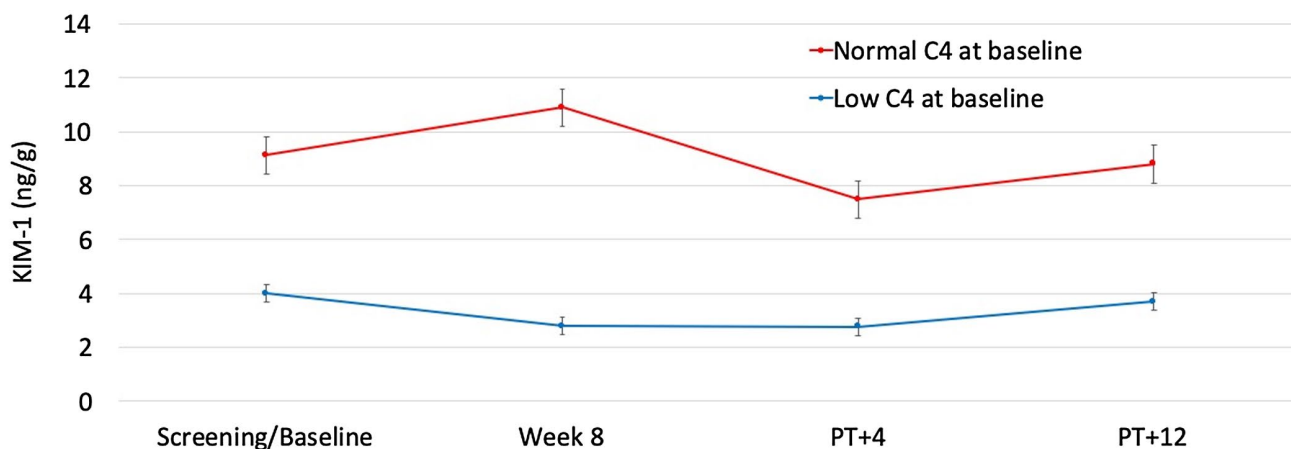

FIG. 8. Average urinary KIM-1 values over the study period, by C4 status. Patients were stratified into two groups: low C4 at baseline and normal C4 at baseline. Mean KIM-1 and standard error bars are shown at four timepoints throughout the course of treatment, stratified by baseline C4 status.
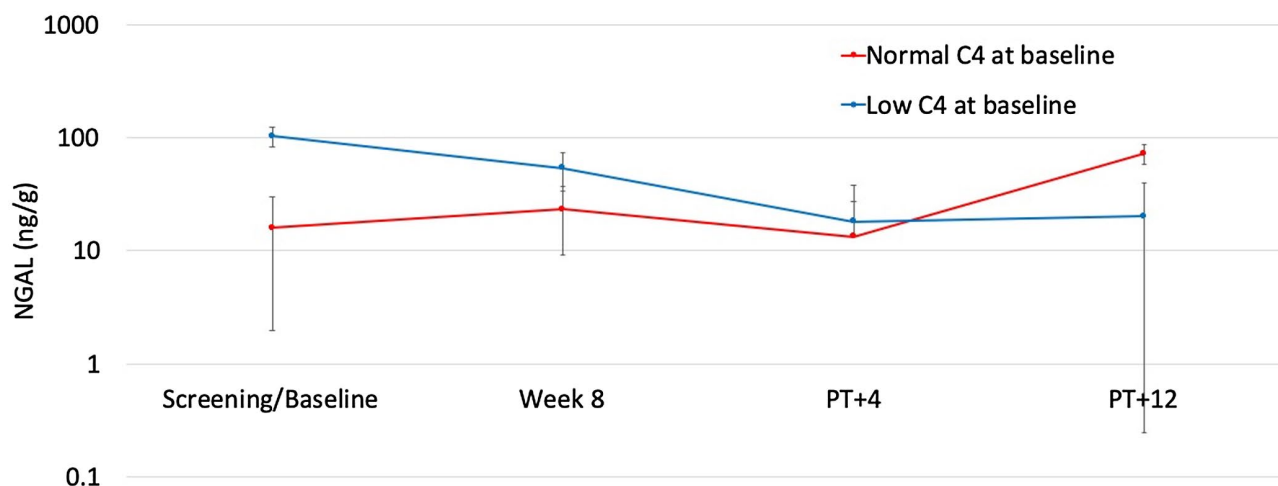
FIG. 9. Average urinary NGAL values over the study period, by C4 status. Patients were stratified into two groups: low C4 at baseline and normal C4 at baseline. Mean NGAL and standard error bars are shown at four timepoints throughout the course of treatment, stratified by baseline C4 status. The y-axis is on a base-10 logarithmic scale in response to skewness toward large values.

after DAA therapy; thus, 50 patients were included. Their baseline characteristics are shown in the right-most column of Table 1. The mean (SD) age was 63.9 years (9.6 years). Most (62%) were male; 48% self-identified as white, non-Hispanic, 30% as black, 14% as Hispanic, and 4% as Asian. Comorbidities were common and included hypertension in 88%, diabetes in 48%, cirrhosis in 62%, and co-infection with human immunodeficiency virus (HIV) in 10%. Distribution of HCV genotypes is also given in Table 1. Average baseline serum creatinine was 1.57 (0.41) mg/dL, corresponding to a eGFR of 45.7 (9.1) mL/min/1.73 m^2. Most patients (96%) received a sofosbuvir-based regimen. Ribavirin was used in only 22%. Only 2 patients (4%) discontinued DAAs early. Forty-two (89%) of the 47 patients with known SVR status were cured. Three patients (6%) did not have a known SVR status based on chart review. Twenty patients (40%) had low C4 level before beginning DAAs. The characteristics of patients with low C4 versus those with normal C4 is given in Supporting Table S4. The rate of hypertension and diabetes was similar among patients with low C4 and normal C4; cirrhosis was more common in patients with low C4. Chart review identified that less than half of those (8 of 20) had clinical signs of mixed cryoglobulinemic syndrome. In the population of patients with CKD who had at least one
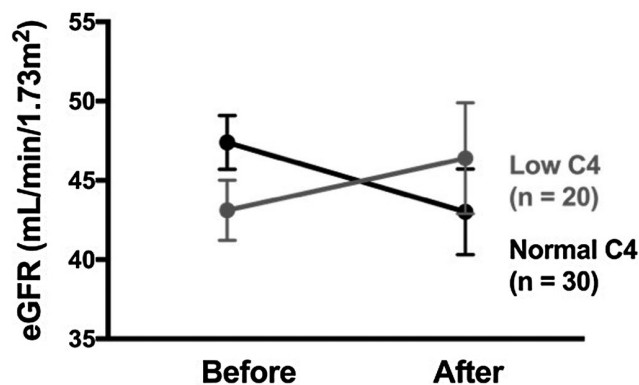
FIG. 10. Average eGFR before and after DAA treatment in 50 patients with CKD, by baseline C4 status. The eGFR of patients with low C4 at baseline was 43.1 mL/min/1.73 m^2 before treatment and increased to 46.4 mL/min/1.73 m^2 after treatment. Patients with normal C4 at baseline had a eGFR of 47.4 mL/min/1.73 m^2 before treatment and 43.1 mL/min/1.73 m^2 after treatment ($P = 0.02$ for differences).

serum creatinine measurement in the first year after completing DAAs (n = 50), we examined the effect of baseline complement levels on change in eGFR (Fig. 10). Low C4 was significantly associated with increase in eGFR after treatment (Table 3). These data suggest that patients with CKD and HCV who have low C4 levels are most likely to have improvement in kidney function with DAAs.

TABLE 3. UNIVARIABLE COMPARISON OF
BASELINE PREDICTORS OF eGFR IMPROVEMENT
IN THE RETROSPECTIVE COHORT

Baseline predictors of eGFR improvement after DAAs	Univariate	Model
	Beta (95% CI)	P Value
Age, per 10 years	–0.10 (–0.4 to 0.4)	0.96
Sex (female vs. male)	–2.87 (–10.1 to 4.4)	0.43
Race (white vs. non-white)	1.96 (–5.1 to 9.0)	0.58
Diabetic vs. nondiabetic	–5.34 (–12.2 to 1.5)	0.12
Cirrhosis vs. noncirrhotic	5.96 (–1.1 to 13.0)	0.096
Hypertension vs. non-hypertensive	0.033 (–10.8 to 10.9)	1.00
HIV coinfection	5.61 (–6.0 to 17.2)	0.97
Previous kidney or liver transplant	5.75 (–2.3 to 13.8)	0.16
Baseline low C4 level	7.73 (0.89 to 14.5)	0.028

Note: Change in eGFR was defined by the difference in eGFR from baseline to posttreatment average in the 12 months after completing DAA therapy.
Abbreviation: CI, confidence interval.

Discussion

In this pilot trial of LDV/SOF in patients with proteinuric CKD, we found that all patients with low complement C4 levels at baseline experienced at least a 25% reduction in proteinuria with DAA therapy. We validated that low C4 status was associated with improvement in eGFR after DAA therapy in a large, real-world cohort of DAA-treated patients in our health care network.[12] Low serum level of complement C4 is a marker of immune complex disease; it is consumed by activation of complement by the classical pathway. This can be trigged by cryoglobulinemia, which may be high grade or low grade and may or may not be associated with other symptoms of mixed cryoglobulinemic syndrome. It is important to note that most patients with low C4 in both the pilot trial and retrospective cohort did not have any clinical features of mixed cryoglobulinemic syndrome. Low C4 level may be a marker of a phenotype of kidney disease that is most likely to respond favorably to DAAs, suggesting that immune complex-mediated glomerular pathology is present.

Our findings are congruent with histopathologic studies in patients with HCV showing that undiagnosed glomerular disease may be common. In a study of thirty kidney biopsies obtained from patients with HCV at the time of liver transplant, 97% had glomerular abnormalities, including 83% with mesangial immune deposits and 53% with mesangial and subendothelial immune deposits.[15] An autopsy study in patients with HCV infection similarly found glomerular disease to be prevalent and often subclinical: 55% had glomerular abnormalities (11% membranoproliferative glomerulonephritis, 18% mesangial proliferative glomerulonephritis, 3% membranous nephropathy, 23% with mesangial thickening), only 18% of whom had had an abnormal urinalysis in the previous year.[16] Taken together, these studies suggest that the prevalence of subclinical glomerular disease in patients with HCV infection may be substantial.

In our retrospective cohort, the 30 patients with CKD who had normal C4 at baseline had a 3.3-mL/min decrease in eGFR from baseline to post treatment. The reason for eGFR decline is unclear but may reflect natural progression of underlying CKD; we have previously reported that unexplained acute kidney injury in patients treated with sofosbuvir-based DAAs is rare.[17] Nevertheless, closer examination of this study population is needed. We note that 1 trial patient had a substantial rise in proteinuria during the study period. Review of this case revealed that during the study period her blood pressure was out of control and she was not meeting glycemic targets for diabetes management. It is worth mentioning that our previous study demonstrated that, on average, patients with diabetes who undergo DAA therapy had increased proteinuria from baseline to post treatment; however, it is unclear whether this is due to natural progression of baseline diabetic nephropathy.[17] This important question requires further study.

Our study has several limitations. Our pilot trial was limited by the small number of patients enrolled. Additionally, 2 subjects experienced virologic relapse, complicating the analysis of kidney function changes in these patients. Thus, the relationships between baseline characteristics and changes in proteinuria are descriptive in nature; nevertheless, we were able to validate the relationship of low C4 levels with changes in kidney function in a separate retrospective cohort of patients with CKD. Our study protocol only approved follow-up of patients for up to 1 year after initiating treatment. Therefore, we are limited by the lack of long-term follow-up that would provide more comprehensive trends. Additionally, given that subjects were only treated with LDV/SOF, the generalizability of this study to patients treated with other DAA regimens is limited. In our retrospective cohort, less than half of the total CKD population had complement C4 levels checked before beginning DAAs, suggesting that this

is a selected population. Additionally, we were unable to evaluate proteinuria in our retrospective cohort, as too few patients had both pretreatment and post-treatment proteinuria checked. Furthermore, a substantial number of patients in the retrospective cohort were transplant recipients, and we did not explore the effect of exposure to nephrotoxic immunosuppression. Both analyses are limited by the absence of a control group. However, using patients whose HCV infection had not yet been treated as a comparison group is problematic, as untreated patients differ in terms of medical comorbidities, insurance status, and other important social determinants of health. In this study, patients served as their own control, comparing their kidney function before and after initiating DAAs and reporting within-patient changes. Finally, this pilot trial did not include patients with eGFR less than 30 mL/min/1.73 m^2; future research will be needed to identify factors that are associated with improved kidney function in patients with advanced CKD.

In conclusion, this study suggests that low C4 might identify patients with HCV and CKD whose kidney disease is likely to improve with DAA treatment. This was the case even for patients with low C4 without overt signs of mixed cryoglobulinemic syndrome. Our observations provide additional rationale for HCV therapy in patients with CKD but require validation in a prospective series with other licensed regimens. Future analyses with longer follow-up will be needed to determine whether treating HCV early in patients with low C4 could decrease the risk of incident and progressive CKD.

REFERENCES

1) Molnar MZ, Alhourani HM, Wall BM, Lu JL, Streja E, Kalantar-Zadeh K, et al. Association of hepatitis C viral infection with incidence and progression of chronic kidney disease in a large cohort of US veterans. Hepatology 2015;61:1495-502.
2) Crook ED, Penumalee S, Gavini B, Filippova K. Hepatitis C is a predictor of poorer renal survival in diabetic patients. Diabetes Care 2005;28:2187-2191.
3) Lucas GM, Jing Y, Sulkowski M, Abraham AG, Estrella MM, Atta MG, et al. Hepatitis C viremia and the risk of chronic kidney disease in HIV-infected individuals. J Infect Dis 2013;208:1240-1249.
4) Lee CM, Lu SN, Hung CH, Tung WC, Wang JH, Tung HD, et al. Hepatitis C virus genotypes in southern Taiwan: prevalence and clinical implications. Trans R Soc Trop Med Hyg 2006;100:767-774.
5) Lucas GM. Association between hepatitis C virus and chronic kidney disease: heterogeneity begets heterogeneity. Kidney Int 2017;92:546-548.
6) Soma J, Saito T, Taguma Y, Chiba S, Sato H, Sugimura K, et al. High prevalence and adverse effect of hepatitis C virus infection in type II diabetic-related nephropathy. J Am Soc Nephrol 2000;11:690-699.
7) Gane E, Lawitz E, Pugatch D, Papatheodoridis G, Brau N, Brown A, et al. Glecaprevir and pibrentasvir in patients with HCV and severe renal impairment. N Engl J Med 2017;377:1448-1455.
8) Roth D, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H Jr., et al. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. Lancet 2015;386:1537-1545.
9) Borgia SM, Dearden J, Yoshida EM, Shafran SD, Brown A, Ben-Ari Z, et al. Sofosbuvir/velpatasvir for 12 weeks in hepatitis C virus-infected patients with end-stage renal disease undergoing dialysis. J Hepatol 2019;71:660-665.
10) Sise ME, Bloom AK, Wisocky J, Lin MV, Gustafson JL, Lundquist AL, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. Hepatology 2016;63:408-417.
11) Sise ME, Backman E, Ortiz GA, Hundemer GL, Ufere NN, Chute DF, et al. Effect of sofosbuvir-based hepatitis C virus therapy on kidney function in patients with CKD. Clin J Am Soc Nephrol 2017;12:1615-1623.
12) Sise ME, Chute DF, Oppong Y, Davis MI, Long JD, Silva ST, et al. Direct-acting antiviral therapy slows kidney function decline in patients with hepatitis C virus infection and chronic kidney disease. Kidney Int 2020;97:193-201.
13) Park M, Hsu CY, Go AS, Feldman HI, Xie D, Zhang X, et al. Urine kidney injury biomarkers and risks of cardiovascular disease events and all-cause death: the CRIC study. Clin J Am Soc Nephrol 2017;12:761-771.
14) Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-612.
15) McGuire BM, Julian BA, Bynon JS Jr., Cook WJ, King SJ, Curtis JJ, et al. Brief communication: glomerulonephritis in patients with hepatitis C cirrhosis undergoing liver transplantation. Ann Intern Med 2006;144:735-741.
16) Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, et al. Glomerulonephritis in autopsy cases with hepatitis C virus infection. Intern Med 1998;37:836-840.
17) Sise ME, Chute DF, Oppong Y, Davis MI, Long JD, Silva ST, et al. Direct-acting antiviral therapy slows kidney function decline in patients with hepatitis C virus infection and chronic kidney disease. Kidney Int 2020;97:193-201.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1528/suppinfo.